HORIBA Scientific

LabSpec 5

USER MANUAL

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CONTENTS

1. Introduction to this manual

This manual is intended to introduce LabSpec 5 to new users of HORIBA Scientific Raman spectrometers, and to act as a reference guide to the many acquisition and analysis functions available within LabSpec 5.

1.1. Use of this manual

This manual must be used in conjunction with the relevant hardware manual accompanying each HORIBA Scientific Raman spectrometer.

It is advisable that specific hardware/software training is provided to new users by either a trained HORIBA Scientific service or application engineer, or by an experienced user within the institution.

Please note that not all systems will have all available accessories and options installed, and thus the screen shots and images in this manual may not directly correspond to software installed with the specific system you are using. If in doubt, please contact your local HORIBA Scientific office or representative to receive more information about available accessories and options.

1.2. Terminology used

For clarity explanations on key terms and conventions used throughout this manual are given below.

Instrument, equipment, system, spectrometer
These terms refer to the main Raman hardware, which typically comprises laser(s), microscope, monochromator(s), and detector(s).

Accessory
This refers to optional devices such as motorized sample stage, or temperature stage.

Dialog window
This refers to a window which appears during the operation/set-up of a specific function. For example, clicking on the Peaks icon opens the Peaks dialog window, which contains a mixture of text/number inputs, buttons and drop down boxes.

Menu
Underlined text (for example, File or Edit) refers to an item in the Menu Bar. The ">" symbol indicates a menu item, or a sub-menu (for example, File > Save, or Edit > Restore).

[button]
Text in square brackets (for example, **[OK]** or **[Cancel]**) refers to a software button in the dialog window currently being described.

{radio button}
Text in curved brackets (for example {Video On} or {Video Off}) refers to a radio button in the dialog window currently being described.

<enter ↵ >
This refers to the keyboard 'enter' or 'return' button.

Multidimensional spectral array
This refers to a spectral 'map' or 'profile', including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes.

1.3. Display of Icons

Please note that there are two icon views used in LabSpec 5, called "Big icon" and "Small icon". In the descriptions throughout this manual the "Big icon" will be displayed first, followed by the "Small icon".

For further information about the icons and how to switch between "Big icon" and "Small icon" display please see section 4.1, page 89.

2. Introduction to LabSpec 5 software

LabSpec 5 is a fully configured data acquisition and analysis software designed for HORIBA Scientific's range of Raman spectrometers and microscopes.

It enables a full range of experiment types including single spectrum acquisition, multidimensional spectral array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes), video image capture and automated multiwell interrogation.

Many commonly used data analysis and processing routines are also available, including peak labelling and fitting, smoothing, noise reduction, baseline subtraction, linear and non-linear filtering and *direct classical least squares* modelling.

LabSpec 5 is also compatible with Microsoft Visual Basic programming, allowing specific acquisition and analysis routines to be written by the user. In addition, third party software routines can call LabSpec 5 through ActiveX modules.

2.1. Instruments controlled by LabSpec 5

A wide range of HORIBA Scientific instruments are controlled and operated by LabSpec 5 software, including the following:

- LabRAM 300 / LabRAM 1B
- LabRAM HR
- LabRAM ARAMIS
- LabRAM INV
- LabRAM IR and IR^2
- XploRA
- XploRA INV
- T64000
- U1000
- HE spectrograph
- InduRAM
- AccuRA
- Modular Raman Systems (including iHR and micro-HR spectrometers)

2.2. Accessories controlled by LabSpec 5

There is a large number of accessories which can be used with the above Raman systems, many of which can be controlled through LabSpec 5 software, where appropriate. The following list shows common accessories which are fully compatible with LabSpec 5.

If you have other accessories you would like to control with LabSpec 5 then please consult your local HORIBA Scientific office, agent or service department for advice.

- Märzhäuser XY and Z motorized stages (including Tango and LStep controllers)
- Linkam Scientific temperature stages with T94 and T95 controllers
- IDS µEye digital cameras
- PI (Physik Instrumente) piezo XY and Z stages

- Stanford Research Systems lock-in amplifiers
- HORIBA Scientific CCD detectors
- HORIBA Scientific InGaAs array and single channel detectors
- Andor Technology CCD, *i*CCD and EMCCD detectors

2.3. General Layout of LabSpec 5 Software

The main LabSpec screen is divided into a number of distinct regions.

Menu bar
Access to standard Microsoft Windows menu functions (such as File, Edit, Help) and specific LabSpec 5 menus for advanced control of data acquisition and analysis functions.

Icon bar
Access to commonly used file, acquisition and data analysis routines. Includes main STOP button which will stop all currently active acquisition and analysis routines.

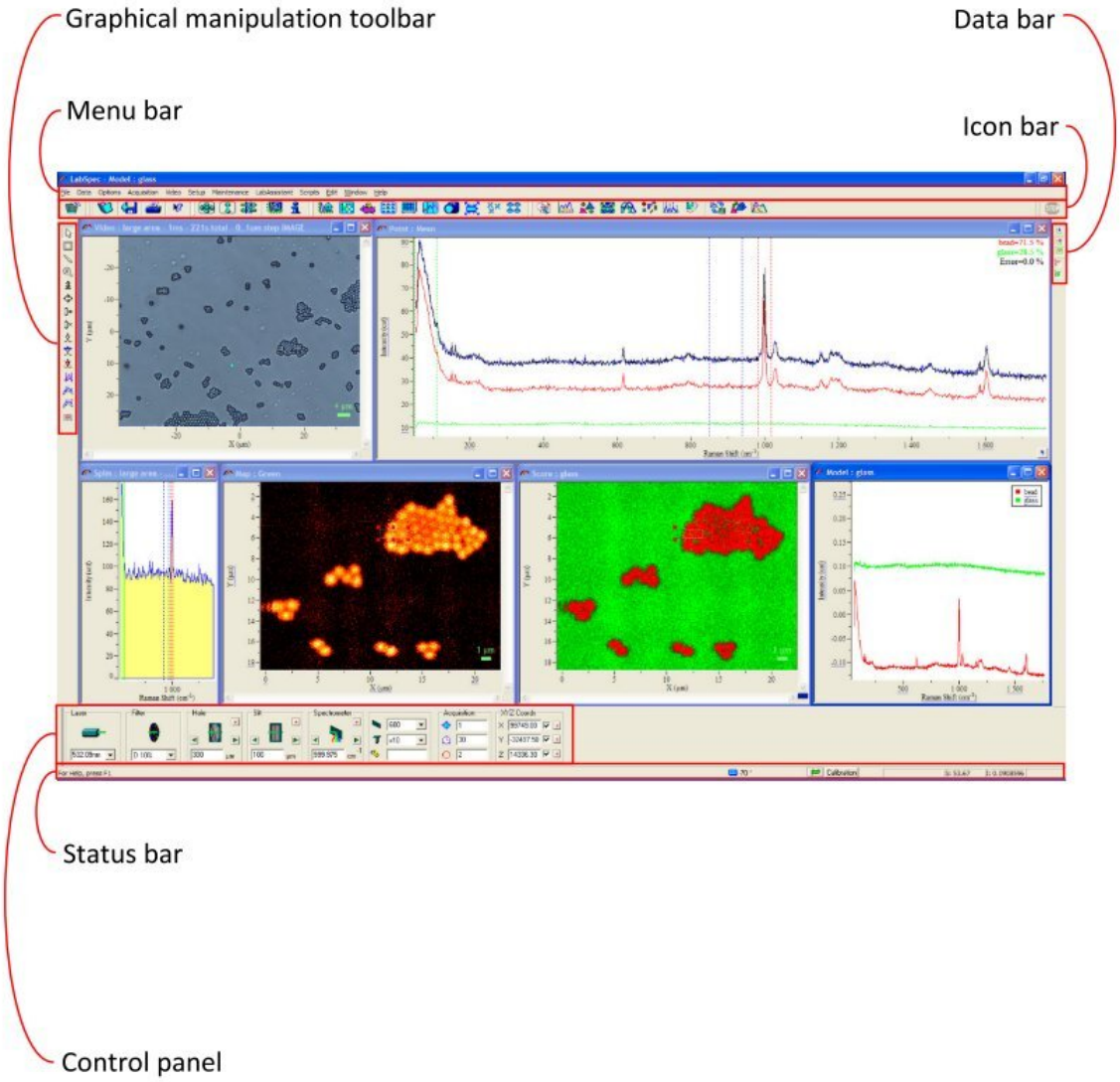
Graphical manipulation toolbar
Access to applicable spectrum/map/image manipulation functions. This is an active toolbar, and its appearance and content will update according to the currently selected window. For example, the options appearing in this toolbar when a spectrum is active will differ from those appearing when a video image is active.

Data bar
List of currently open data (including spectra, profiles, maps and images) allowing specific items to be activated and processed as desired.

Control panel
Access to instrument and accessory hardware controls, and main acquisition parameters (acquisition time, number of accumulations etc).

Status bar
Information about system status (e.g., progress of an acquisition), active options (e.g., intensity correction, or extended range settings), and cursor positions.

Right mouse click menu
Access to applicable spectrum/map/image manipulation functions. This is an active menu, and its appearance and content will update according to the window with which it is being used. For example, the right click menu appearing for a spectrum will differ from that appearing for a video image.

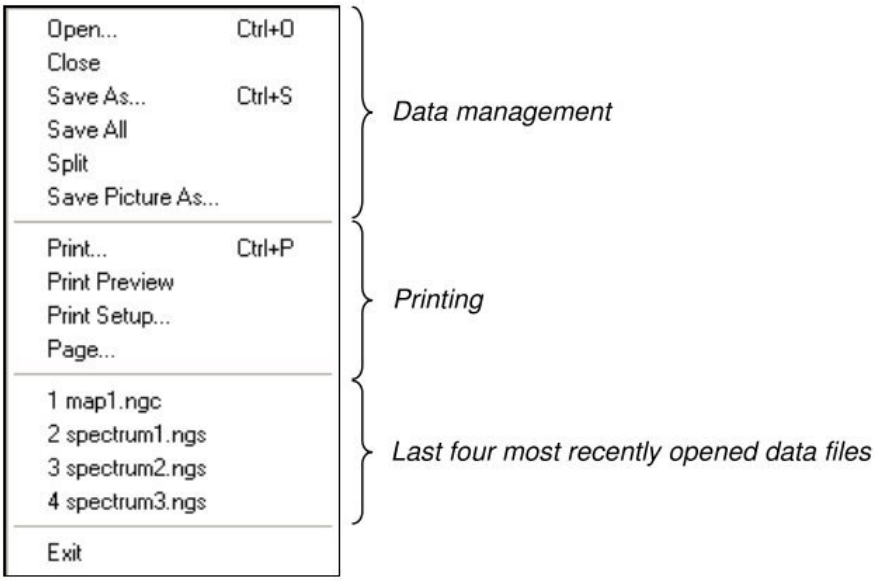


3. Menu Bar

The Menu Bar located at the top of the screen is used to control either standard Windows functions (such as File Open, File Close) or to provide access to specialized data acquisition/analysis modules and options. The Menu Bar will *only* show sections which apply to the correctly installed and configured hardware/software options for your instrument. The image below shows the default sections, but be aware that some of these may not be visible in your software, and others could be visible for specific instrument configurations.

3.1. File

The File menu includes functions for data management (e.g., save or open files) and printing, and in addition includes a list of the last four data files which were opened.



3.1.1. Data Management

Please note that the term 'data file' or 'data object' means any form of data compatible with LabSpec 5, such as spectra, multidimensional spectral arrays (profiles, maps) and video images.

3.1.1.1. File Formats

LabSpec 5 is compatible with a number of different file formats, which will be available to you when opening and saving data. More information about specific options available when saving into these file formats can be found in section 3.1.1.2, page 11. The available file formats are listed below.

.ngs LabSpec 5 spectrum file format.

.ngc	LabSpec 5 array file format, for multidimensional spectral data such as 1D profiles (depth, time, temperature), 2D maps (XY map, XZ and YZ slices) and 3D datacubes (XYZ cubes).
.ngv	LabSpec 5 video camera snapshot image
.tsf	Previous generation LabSpec4 spectrum file format.
.tvf	Previous generation LabSpec4 video and array file format, for multidimensional spectral data such as 1D profiles (depth, time, temperature), 2D maps (XY map, XZ and YZ slices) and 3D datacubes (XYZ cubes).
.ms0, .ss0	Legacy file formats for Dilor Raman instruments
.spc	Grams SpectraCalc spectrum file format, compatible with many common spectroscopy software packages.
.tif	Tiff image file format
.txt	Text file format, compatible with many common spectroscopy software packages, data analysis software packages and spreadsheet software packages.
.bmp, .jpg	Bitmap and JPEG image file formats

3.1.1.2. Data Management Functions

Open...	Open a previously saved data file. This function can be performed using the <CTRL>+O keyboard shortcut.
Close	Close the data file currently active in LabSpec 5.
Save As...	Save the data file currently active in LabSpec 5. See Options for Saving Data (section 3.1.1.3, page 12) for further information. This function can be performed using the <CTRL>+S keyboard shortcut.
Save All	Save *all* open data files of a particular type (spectrum, map, video image) into a single file. For example, if there are three spectra open (spectrum1.ngs, spectrum2.ngs, spectrum3.ngs) it is possible to save a single data file (all_spectra.ngs) which contains the three spectra. When you open the single data file (all_spectra.ngs) the three spectra will be available in LabSpec 5, and can be processed and analysed as single spectra again.
Split	Splits and saves a multidimensional spectrum array (such as a profile or map) into constituent single spectra. Each spectrum will be identified by a coordinate corresponding to its position within the original array data set – for example, spectrum_0_0, spectrum_0_1, spectrum_0_2...spectrum_1_0, spectrum_1_1...

The Split function is compatible with any of the spectrum file formats described above (i.e., .ngs, .tsf, .ms0, .spc, .txt).

Save Picture As... Saves the active LabSpec 5 window as a colour image in .wmf format. Saving a spectrum in this format results in a color image of the spectrum window, including the intensity/spectral axis values and titles. Saving a Raman map image in this format results in a color image of the Raman map including X,Y or Z axes with values and titles, scale marker and intensity scale with values and titles.

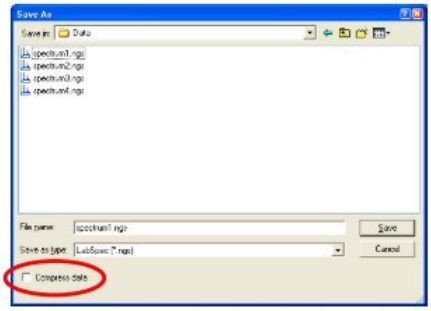
3.1.1.3. Options for Saving Data

Depending on the chosen file format there are a number of additional save options which are set out below.

Note that if a file is saved in a LabSpec format then instrument and measurement parameters will be included as meta data within the file – such meta data can be recalled at any time within LabSpec 5, allowing quick access to previous measurement conditions. Data saved into other file formats will lose this meta data.

LabSpec 5 files (.ngs / .ngc / .ngv)

Select "Compress Data" in order to compress the data files for spectra (.ngs), mapped profiles/images (.ngc) and camera images (.ngv). This will result in smaller file sizes, and ensure that less hard disk storage space is required. There is no loss in quality/information when files are compressed.



Grams Spectracalc (.spc)

Select ""Old" Format" to save the file to be compatible with legacy "old" format Grams software.

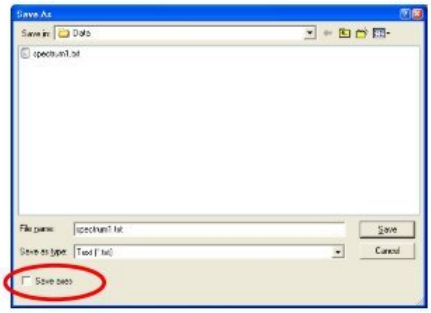
Select "Arbitrary Y unit" to save the spectrum with the Y (intensity) axis labelled as "Arbitrary Y" (instead of "counts").

Select "Even spaced" to save the spectrum with even X axis spacing between data points.

Select "Spectral X unit" to force a spectrum which has "Arbitrary Units" for the X axis to be saved with units of either "Raman shift (cm^{-1})" or "Nanometers" (depending on the units currently selected within LabSpec).

Text (.txt)

Select "Save axes" to save both X and Y axis data in text format. If this item is not ticked, then only the Y axis (intensity) data will be saved in text format.



3.1.2. Printing Functions

Print… Print the current data window (to the default printer) using the Print Template Page. Full information about using and setting up the Print Template can be found in section 3.1.2.1, page 13.

This function can be performed using the <CTRL>+P keyboard shortcut.

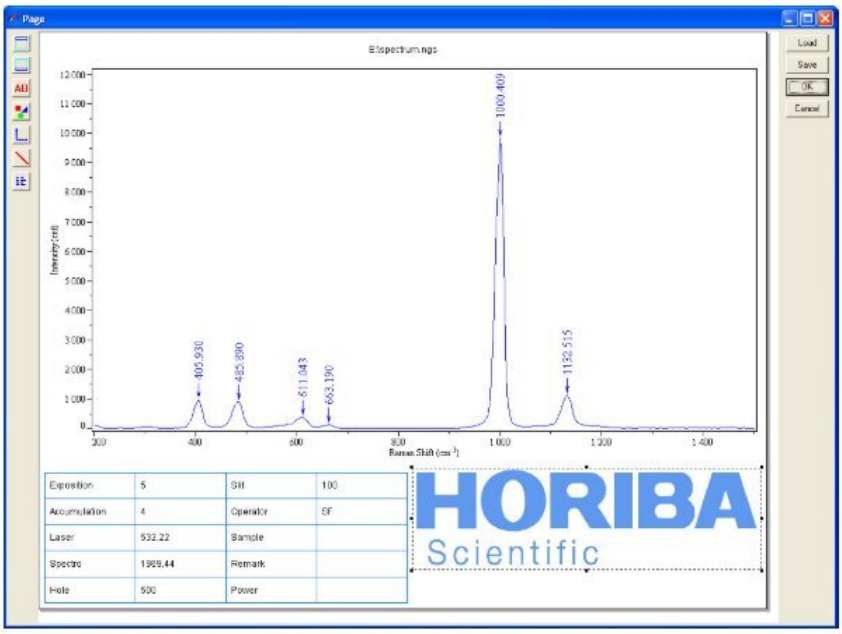
Print Preview Open a preview window showing the Print Template Page (including data from the active data window) as it will be printed.

Print Setup… Open the standard print dialog window to select a printer, and set up print options.

Page… Open the Print Template Page set up dialog window. See section 3.1.2.1, page 13 for full information about setting up the Print Template Page.

3.1.2.1. Print Template Page Set Up Dialog Window

The Print Template Page set up dialog window allows the layout and display information of the Print Template Page to be configured. The Print Template Page is used whenever data is printed from directly within LabSpec 5.



Click on **[Save]** to save the current Print Template Page (in .ini format), using the standard File Save dialog window.

Click on **[Load]** to open a previously saved Print Template Page (in .ini format), using the standard File Open dialog window.

3.1.2.1.1. Print Template Page Elements

The Print Template Page can be edited and organized by the user. It is composed of the following elements, which can be inserted by left clicking and dragging after activating the appropriate icon. Elements can be edited by double clicking or right clicking (see section 3.1.2.1.2, page 15).

Header

Text box in the header position (top of window) displaying either custom text, or data object acquisition date, name, file path, or associated user name.

Footer

Text box in the footer position (bottom of window) displaying either custom text, or data object acquisition date, name, file path, or associated user name.

Text

Text box which can be positioned freely within the Print Template Page, displaying either custom text, or data object acquisition date, name, file path, or associated user name.

Image

Image display box which can be freely positioned within the Print Template Page. Note that the default image is the HORIBA Jobin Yvon logo, but a standard file browse window will be opened by double clicking on the image display box, allowing other images to be displayed.

Graph

Display box for the active LabSpec 5 data window which can be freely positioned within the Print Template Page. The data and axes will be displayed as set in the main LabSpec 5 graphical user interface (GUI).

Figure

Text box which can be positioned freely within the Print Template Page, displaying either custom text, or data object acquisition date, name, file path, or associated user name.

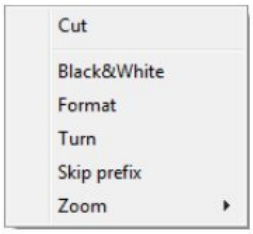
Param

Multiple column text box which can be positioned freely within the Print Template Page, displaying acquisition parameters for the active data object in the active data window. The parameters which are displayed, and their labels, can be customized by double clicking on the parameters text box.

3.1.2.1.2. Editing Print Template Page Elements

Right Click Menu

Right click anywhere on the Print Template Page to display the right click menu.



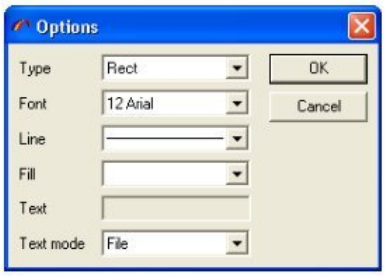
- Select "Cut" to delete the selected element on the Print Template Page.
- Select "Black&White" to display the Print Template Page in greyscale.
- Select "Format" to open the default printer Page Setup dialog window, allowing control of page layout (portrait or landscape), paper source, and print margins.
- Select "Turn" to toggle between landscape and portrait page views.
- Select "Skip prefix" to hide the data object 'type' prefix from the print job. This is particularly useful when printing the document to pdf, where typically the

print job name will be "<Object Type>: <Object Name>" (e.g., "Spectrum: data001"). When "Skip Prefix" is ticked, the print job name will be the object name only (e.g., "data001").

- Select "Zoom" to change the display size of the Print Template Page.

Header, footer, text and figure display boxes

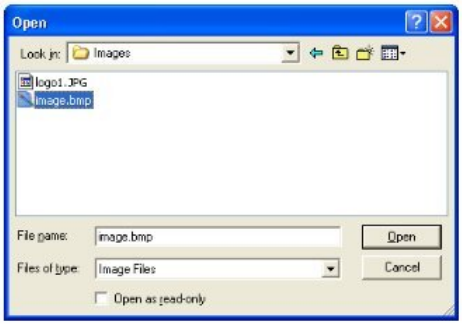
Double click on the box to display the Options dialog window. The box can be configured using the following controls:



- Type – select the box shape from the "Type" drop down box.
- Font – select the font, style, size and color from the "Font" drop down box.
- Line – select the box outline color, style and width from the "Line" drop down box.
- Fill – select the box fill color and style from the "Fill" drop down box.
- Text – if custom text is selected from the "Text mode" drop down box, type in the desired text in the "Text" box.
- Text mode – select the text information which will be displayed in the box, from the "Text mode" drop down box. Choose to display custom text, or data object acquisition date, name, file path, or associated user name.

Image box

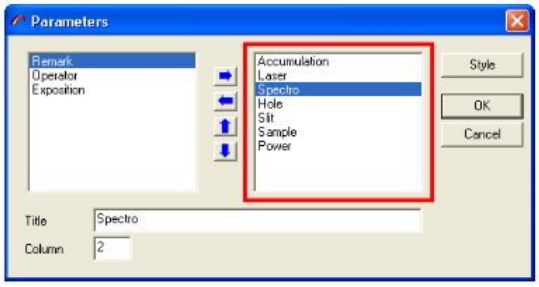
Double click on an image box to display a standard File Open dialog window. Browse to locate the image which will be displayed in the image box.



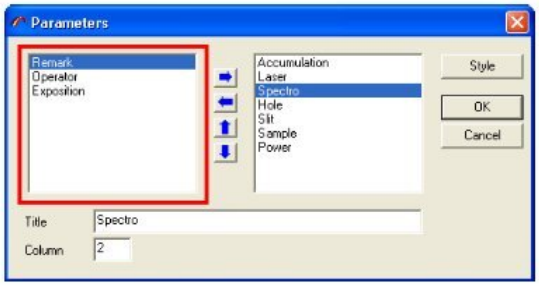
Parameters box

Double click on a parameters box to display the Parameter Selection dialog window.

Parameters which will be displayed in the parameters box on the Print Template Page are shown in the right hand list box.

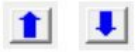


Other available parameters (from the standard parameters information window – see section 4.4.5, page 92) are shown in the left hand list box.



Click on the left and right arrow icons to move parameters from one list box to the other.

Click on the up and down arrow icons to adjust display order of parameters in the parameters box.



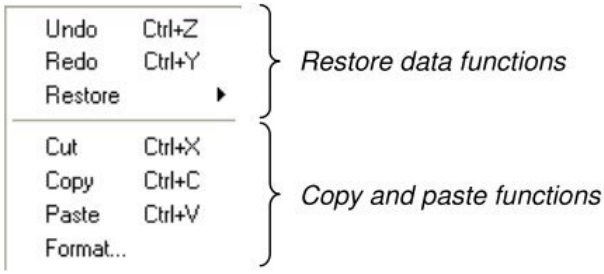
The Title text box shows the display name for the parameter selected in the right hand list box. The display name can be modified by typing the desired name in the Title text box.

Click on **[Style]** to open the Style Options dialog window – the options displayed here are identical to those listed above for header, footer, figure and text boxes.

In the Column text box type in the number of columns in which the parameters will be displayed. Note that a single column is a double-column entity, in order to display the parameter name and value.

3.2. Edit

The Edit menu includes functions to restore data to its original form after it has been manipulated, and to copy and paste data.



3.2.1. Restore Data Functions

Undo — Undo the last action which has been made in LabSpec 5. For example, if a baseline subtraction does not give the desired result, the Edit > Undo function will return the spectrum to its original form.

This function can be performed using the <CTRL>+Z keyboard shortcut.

Redo — Redo the last action which has been undone. For example, if a process has been reversed using Edit > Undo, it can be repeated using the Edit > Redo function.

This function can be performed using the <CTRL>+Y keyboard shortcut.

Restore — Reload a data object which has been closed using the Delete icon (see section 4.2, page 90). Edit > Restore will display a list of all data objects which have been deleted – left click on the data object name to reload it.

3.2.2. Copy and Paste Functions

Cut — Close the currently active data file so that it is no longer displayed in LabSpec 5. Note that if the data has been saved to the computer hard drive, then it will remain there after it has been cut from LabSpec 5. The data can be re-opened using File > Open.

If data has not been saved, or has been modified since the last save, a warning message will appear prompting for you to save the changes. Click **[Yes]** to save the changes, or **[No]** to close the data without saving the changes.

Real Time Display (RTD) data (see section 4.5.1, page 95) will be closed without a prompt to save the changes.

This function can be performed using the <CTRL>+X keyboard shortcut.

Copy — Copy the currently active data to the clipboard, so that it can be transferred to a different LabSpec data window, or to a different software program. Please also see information below about the copy/paste format, which allows you to control how the data is copied.

This function can be performed using the <CTRL>+C keyboard shortcut.

Paste — Paste the data from the clipboard into LabSpec 5 or into a different software program.

This function can be performed using the <CTRL>+V keyboard shortcut.

Format... — Control the format for the copy and paste functions.

Data – this allows copy/paste of data in its LabSpec 5 format, which includes both the actual data and its 'type' designation. Common data types within LabSpec 5 include "spectrum", "map point spectrum", "map model spectrum", and "profile". If data is copied or pasted as "Data", then it retains this

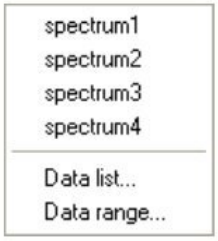
designation. Note that data of a certain 'type' can *only* be pasted into its matching 'type' window. For example, "spectrum" data can only be pasted into the "spectrum" window, and it is not possible to paste it into a "map point spectrum" window. "Data" copy/paste functions are intended to be used within LabSpec 5 only.

Text – this allows copy/paste of data in text format only, which includes actual data but excludes its 'type' designation. In this case, it is possible to paste data from one 'type' window into another, and is a useful method to transfer data into different windows. For example, a "map point spectrum" can be copied and pasted into a "spectrum" window.

Picture – this allows a screen capture of the currently active window, thus allowing an image of the window to be pasted into other software. If data is copied as a picture then it can be pasted as a picture only.

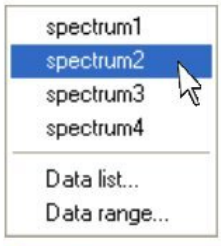
3.3. Data

The Data menu displays a list of the first eight open data files, and allows you to open a full list of all data files, and to view and edit parameters relating to the spatial/spectral/intensity range of the currently active data.

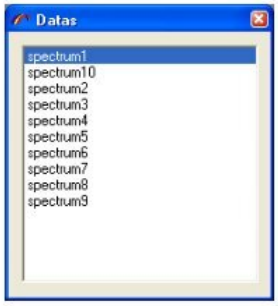


3.3.1. Selecting Data

An open data file can be selected (activated) by clicking on the file name displayed in the Data menu.



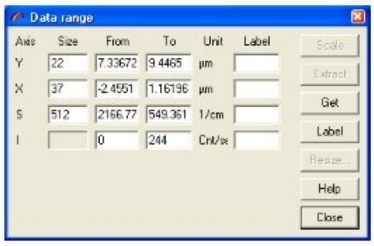
If more than eight data files are open then the full list can be displayed by clicking on "Data list...". Click on the file name to select (activate) the file. The data list will automatically close once the chosen file has been selected.



Data files can also be selected by clicking on coloured radio button in the data bar (see section

6, page 195), or by using the SHIFT+click function on the spectrum in the spectrum display window.

3.3.2. Data Range



Clicking on Data > Data range... opens the "Data range" dialog window, which displays information about the number of data points and maximum and minimum values for the active data. Typical information includes maximum and minimum values for the spectrum axis, intensity axis, XYZ spatial axes, and colors. The exact categories which are displayed depend on the specific 'type' of the selected data.

3.3.2.1. Data Range Information

The "Data range" dialog window shows the following information:

Axis	Identification of the axis type (see section 3.3.2.2, page 20).
Size	Number of data points within the axis.
From / To	The end limit values ('start' and 'stop') for the axis.
Unit	The units associated with the axis.
Label	The display label for the axis. Note that default labels (such as "Raman shift (cm^{-1})" for the X axis of a spectrum) are not displayed.

3.3.2.2. Data Range Axes

The "Data range" dialog window shows information about the following data axes:

Spectrum axis (S)
This will be displayed for spectral data, showing the information for the Raman shift (cm^{-1}) or wavelength (nm) axis.

Intensity axis (I)
This will be displayed for spectral data, Raman XYZ maps/slices/profiles and optical images, showing the information regarding the intensity axis. The units will be counts (cnt) or counts per second (cnt/sec) for spectra and Raman XYZ maps/slices/profiles, and arbitrary units (a.u.) for optical images.

Spatial axes (X, Y and Z)
These will be displayed for Raman XYZ maps/slices/profiles, and optical images, with units of micrometers (µm).

Colors (C)
These will be displayed for optical images only, and shows the number of colors associated with the image. Typically there will be three colors (red, green and blue) for the image.

3.3.2.3. Data Range Functions

Scale — Allows the axis scale to be modified (for example, shifted in position, or stretched/compressed). To use this function, manually adjust the figures in the "From" and "To" boxes, and then click **[Scale]**. The axis will be scaled using the new "From" and "To" values. This function can be used to shift a spectrum to higher or lower Raman shift (cm^{-1}) or wavelength (nm) position. In this case, ensure that the "From" and "To" values are adjusted by exactly the same amount; otherwise the spectrum will be stretched or compressed.

Extract — Truncates the data file by deleting all data outside the "From" and "To" values. To use this function, manually adjust the figures in the "From" and "To" boxes, and then click **[Extract]**. This function can be used in conjunction with **[Get]** (see below) to extract just the currently displayed data.

Get — Updates the Data Range values according to the current display in the active spectrum/map/image window. To use this function click **[Get]** to refresh the Data Range window with the current display values. This function can be used in conjunction with **[Extract]** (see above) to extract just the currently displayed data. For example, use the Zoom tool (see section 5.5, page 168) to select the desired data range, and then click on **[Get]** and then **[Extract]**.

Label — Re-labels the data axes according to the values set in the "Label" boxes. Note that default labels (such as "Raman shift (cm^{-1})" for a spectrum X axis) are not displayed in the "Label" boxes. To use this function type the desired axis label in the relevant "Label" box and then click on **[Label]**.

Resize... — Modifies the number of data points for an axis. For example, the "Resize..." function allows a spectrum of 1024 data points to be reduced to 512, or an image of 100 x 100 data points to be increased to 150 x 150.

To use this function manually modify the number of data points in the "Size" box, and then click on **[Resize...]**.

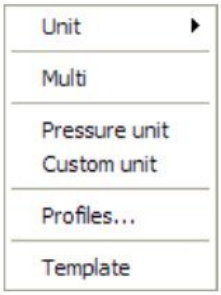
Two functions are available for "Resize...".

Polynom – fits a polynomial curve through a number of adjacent pixels, and calculates the required new data points based on this curve. The degree of the polynomial and the number of adjacent pixels can be selected from the "Poly degree" and "Pixel number" drop down boxes.

Bin – co-adds a specified number of adjacent pixels. The binning factor can be selected from the drop down box – this indicates the number of adjacent pixels which will be co-added. For example, binning factor=2 indicates that every two pixels will be binned (i.e., 1+2, 3+4, 5+6...).

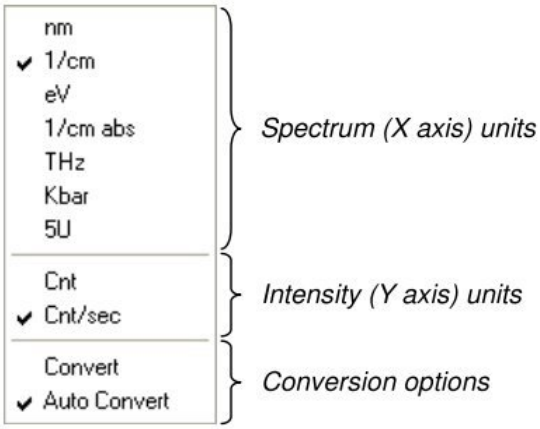
3.4. Options

The Options menu allows control of a number of optional functions within LabSpec. Note that many items in the Options menu will only be visible if they have been specifically activated, and hence the visible menu may vary from that shown below.



3.4.1. Unit

Hovering the mouse over Options > Unit a new menu will be displayed, allowing you to select the units for the spectrum and intensity axes. A tick indicates which option is currently selected and active.



3.4.1.1. Spectrum (X axis) Units

nm	Nanometers (nm)
1/cm	Raman shift (cm^{-1}) – energy (in wavenumbers, cm^{-1}) relative to the laser line
eV	Electron volts (eV)
1/cm abs	Absolute wavenumbers (cm^{-1}) – absolute energy ($cm^{-1} = 10^7/nm$)
THz	Terahertz frequency (THz) – ($THz = 0.03cm^{-1}$)
kbar	Kilobar (kbar) [Only visible if "Pressure units (custom units)" module is active within LabSpec]
5U	Position of user defined custom units – the unit name (e.g., 5U) will be displayed as set by the user. [Only visible if "Pressure units (custom units)" module is active within LabSpec]

3.4.1.2. Intensity (Y axis) Units

cnt	Counts
cnt/sec	Counts per second

3.4.1.3. Conversion Options

Convert	Click on "Convert" to convert the relevant axis to the selected units.
Auto Convert	When "Auto Convert" is ticked the units for the relevant axis are automatically converted when the units are modified.

3.4.2. Multi

The "Multi" function allows batch processing of open data files.

If "Multi" is ticked then when a data processing function (e.g., smoothing or peak searching) is started it will be applied to all data files open within the active window.

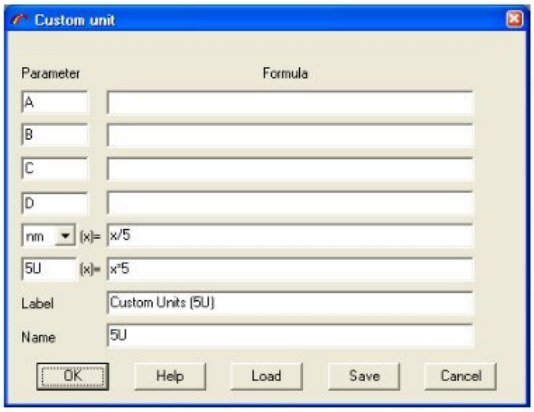
If "Multi" is used in conjunction with File > Save As, then all of the data within the active window will be saved sequentially. The files will be saved in the order they are presented within the Data Bar (see section 6, page 195) or Data List (see section 3.3, page 19).

3.4.3. Pressure Unit

The "Pressure Unit" dialog window allows control of the formulae used to convert data into pressure units (such as kilobar, kbar). The dialog window and its use is identical to the "Custom Unit" dialog window, which is described in section 3.4.4, page 24.

3.4.4. Custom Unit

The "Custom Unit" dialog window allows specific spectral (X axis) units to be defined and used. Click on "Custom Unit" to open the following window, which includes a number of sections:



Constants A, B, C and D

Conversion formula of custom unit to standard unit (nm or cnt) *and* vice versa

Label and name for specified custom unit

Custom units are defined by inserting an appropriate mathematical formula to convert from standard units (of nanometers, nm, or counts, cnt). In the example shown in the above image, the custom units 5U are calculated as 5U = x * 5, where x is nm. The reverse conversion is also shown, so that nm = x / 5, where x is now 5U.

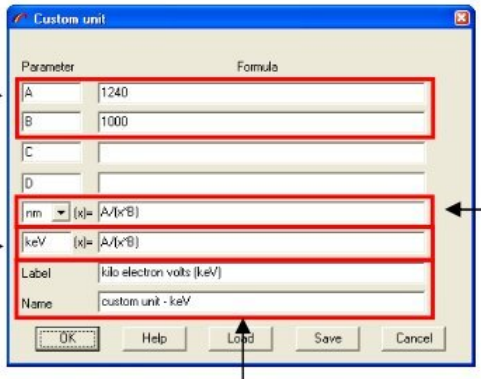
3.4.4.1. Defining Custom Units

In order to define custom units it is necessary to have mathematical formulae which relate the custom unit to standard units (of nanometers, nm, or counts, cnt). Formulae are required which convert (a) the standard unit to custom unit, and (b) the custom unit to standard unit.

The following example of converting nanometers (nm) to kilo electron volts (keV) will illustrate the principles of creating custom units.

The formula for converting nanometers (nm) to kilo electron volts (keV) is: keV = 1240 / (nm * 1000)

The formula for converting kilo electron volts (keV) to nanometers (nm) is: nm = 1240 / (keV * 1000)



Set constant values for A and B

Specify the custom unit (which will be displayed in Options > Unit) as "keV" and type in the formula to convert from nm to keV

Choose the standard unit (in this case nanometers "nm") from the drop down box, and type in the formula to convert from keV to nm.

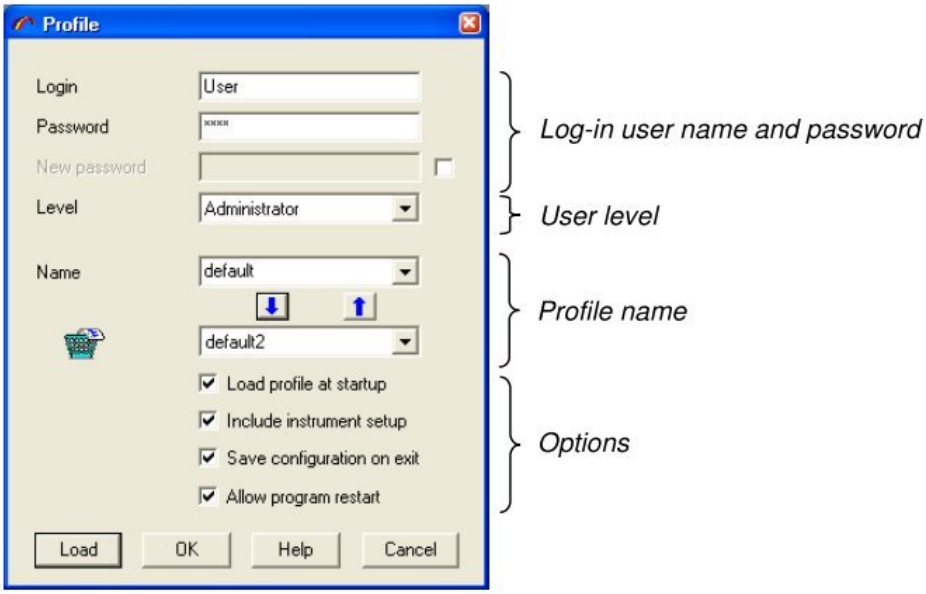
Type in the "Label" which will be used as the axis label for the custom unit, and the "Name" for this specific custom unit configuration.

The configuration can be saved (as a .ini file) by clicking on **[Save]**, and previously saved configurations can be opened by clicking on **[Load]**.

3.4.5. Profiles...

The "Profiles..." module allows individual user accounts to be set up, so that personal settings and the user's last used instrument configuration can be restored. If desired, it can configured to prompt for a user name and password when the software is opened.

Please note that "Profiles..." is not intended to be a software security module, but rather a method of maintaining ease of use in a multi-user environment. The module allows each user to have LabSpec configured in a different way – in addition, it can be used to prevent confusion with acquisition/analysis settings, by ensuring that all settings are restored to the user's preferred values.



Log-in User Name and Password
Each user has an individual log-in user name and password

User Level
Three user levels are available: (1) administrator, (2) operator and (3) guest.

- "Administrator" has full control of the software and system.
- "Operator" cannot modify instrument parameters (such as laser wavelength, confocal hole, slit etc) but can control acquisition time and number of accumulations, and has full access to data processing functions.
- "Guest" has full control of the software and system, but is intended for one-off guest users who do not have their own user name and password.

Profile name
Each user can have multiple profiles associated with his/her account. This allows the user to choose a specific preferred configuration at log-in.

Options

The options set how the "Profiles..." module operates. Note that the displayed settings are used for all users; they are not saved individually as part of the user's profile.

- Load profile at start up – when this box is ticked user log-in is required when the software is opened. If this option is not active, then users can log-in *via* Options > Profiles...
- Include instrument set up – when this box is ticked the instrument settings (such as laser, confocal hole, slit, spectrometer position etc) will be saved as part of the user's profiles. This function ensures that a user will see either his/her preferred instrument configuration, or the instrument configuration that (s)he last used.
- Save configuration on exit – when this box is ticked the instrument and software configuration will be automatically saved when the software is closed. This means that when the user logs in again, the instrument and software will be restored to his/her last used configuration.
- Allow program restart – when this box is ticked, the software is permitted to close and re-open when a new user profile is selected *via* Options > Profiles... In this way it is possible to apply the user's desired instrument and software configuration.

3.4.5.1. User Log-in

3.4.5.1.1. Log-in At Start of LabSpec

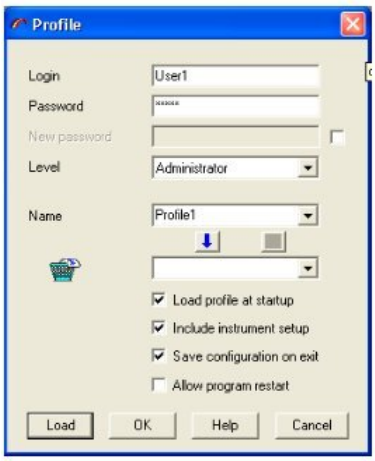
If the "Load profile at start up" is ticked (see 3.4.5, page 25) a window will appear prompting for "Login" and "Password" when LabSpec is started. Type in the correct log-in user name and password, and then select the desired profile from the drop down box.

Click **[Load]** to apply the profile.

Click **[Skip]** to access LabSpec at an "Operator" level, without having to log-in. Note that an "Operator" cannot modify instrument parameters (such as laser wavelength, confocal hole, slit etc) but can control acquisition time and number of accumulations, and has full access to data processing functions.

3.4.5.1.2. Log-in During a LabSpec Session

If the "Load profile at start up" is not ticked, or if you wish to change profile during a LabSpec session use Options > Profiles... Type in the correct log-in user name and password, and then select the desired profile from the "Name" drop down box.

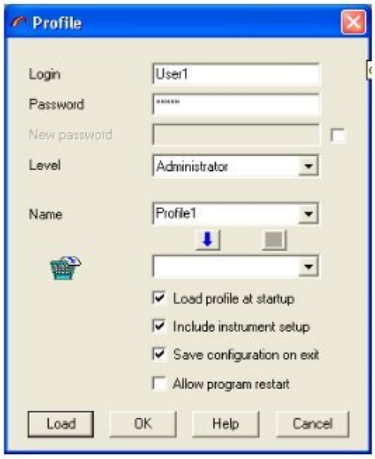


Click **[Load]** to apply the profile.

Note that if "Allow program restart" is ticked (see section 3.4.5, page 25) then LabSpec will be closed and re-started automatically.

3.4.5.2. Creating a New User Profile

New user profiles are created in the Profiles window (Options > Profiles...), by specifying the user name, password, level and profile name(s). The new user profile will be created when you next exit LabSpec.



"Login" User Name
Type in the new "Login" user name.

Password
Type in a password in the "Password" box.

Profile "Name"
Type in a name for this specific profile into the "Name" drop down box. Each user can specify multiple profiles activated with a single "Login" user name and password.

Level
Select the appropriate user level from the "Level" drop down box.

Three user levels are available: (1) administrator, (2) operator and (3) guest.

- "Administrator" has full control of the software and system.
- "Operator" cannot modify instrument parameters (such as laser wavelength, confocal hole, slit etc) but can control acquisition time and number of

accumulations, and has full access to data processing functions.

- "Guest" has full control of the software and system, but is intended for one-off guest users who do not have their own user name and password.

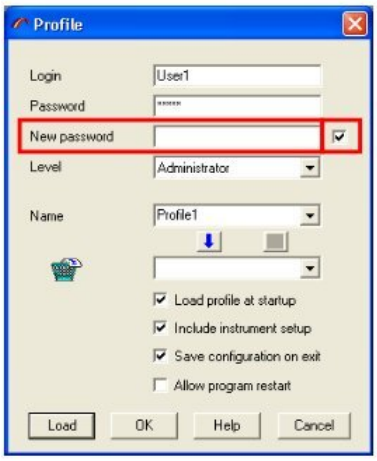
Options

Note that the Profile "Options" (see section 3.4.5, page 25) are universally applied for *all* users, and cannot be independently set for each user.

3.4.5.3. Changing a User Profile Password

To change an existing password, select Options > Profiles..., and type in your "login" user name and "password". Type the new password into the "New Password" box. If the "New Password" box is greyed out and inactive, tick the box to the right hand side to activate it.

Click **[OK]** to register the new password. This password will be active from the next log-in.



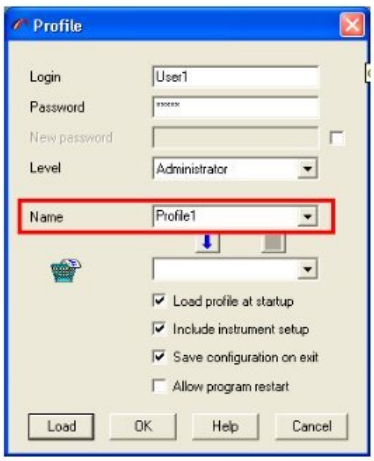
3.4.5.4. Using Multiple Profiles With a Single User

Each user can save multiple profiles activated with a single "Login" user name and password.

3.4.5.4.1. Creating a New Profile Name

In Options > Profiles... log-in with your "Login" user name and password.

In the "Name" drop down box type in the desired name for the new profile and click **[OK]** to register the new profile name. The new profile will be created and saved when you next exit LabSpec.



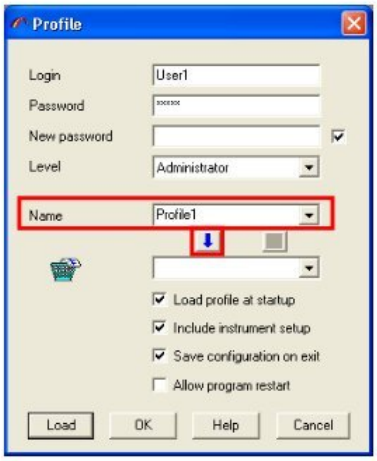
3.4.5.4.2. Deleting a Profile Name

In Options > Profiles... log-in with your "Login" user name and password.

In the "Name" drop down box select the name of the profile you wish to delete, and then click on the blue 'down arrow' to move the selected profile name to the trash bin.

Click **[OK]** to exit the Profiles... dialog window.

Note that deleted profiles are always available to be restored to active use. See *Restoring a Deleted Profile Name* below.

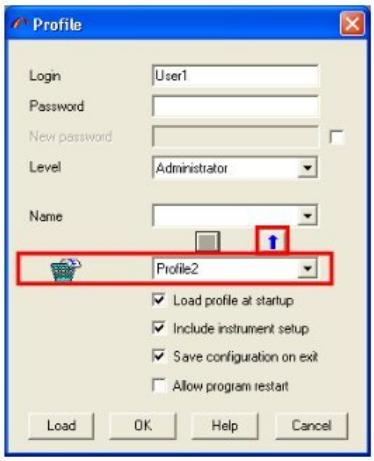


3.4.5.4.3. Restoring a Deleted Profile Name

In Options > Profiles... log-in with your "Login" user name and password.

In the trash can drop down box select the name of the profile you wish to restore, and then click on the blue 'up arrow' to restore the deleted profile name to the active "Name" drop down box.

Click **[OK]** to exit the Profiles... dialog window.



3.4.6. Template

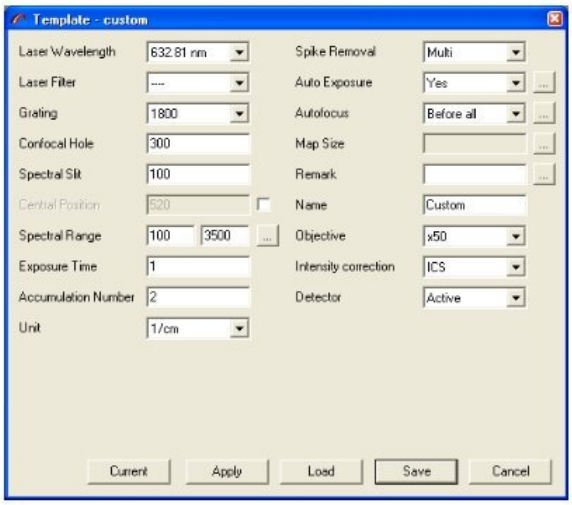
The "Template" module allows you to save a specific instrument configuration and measurement set up as a template. The template can be recalled and applied as required. Templates are useful when you wish to measure a number of sample types, each of which requires a specific measurement configuration. In this case, a number of templates can be set up allowing you to quickly recall the desired configuration without needing to set each parameter individually.

To learn more about the various options shown in the template dialog window please consult the relevant section of this manual.

Enhanced template control is provided through the LabAssistant menu (see section 3.8, page 76), and it is recommended that the LabAssistant function is used in preference to Options > Template.

3.4.6.1. Using Templates

Open the "Template" dialog window by clicking on Options > Template.



In the "Template" dialog window set up the instrument and measurement configuration as required. Note that all modules and accessories active on the system will be visible within the "Template" window. Depending on your instrument configuration the "Template" window you see may vary from that shown here.

Click on **[Current]** to import the current instrument configuration and measurement set up into the Template.

Click **[Save]** and **[Load]** to save and open template files (.tpl format) respectively. Standard Windows save and open dialog windows will be displayed.

Click **[Apply]** to apply the current template – motorized hardware changes will be made automatically; manual hardware changes will prompt for you to change the hardware.

3.5. Acquisition

The Acquisition menu allows control of a number of functions which relate to data acquisition.

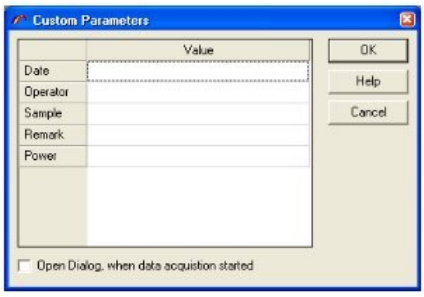
3.5.1. Custom Info

The "Custom info" module allows you to add specific information and metadata to a data file. LabSpec 5 automatically saves hardware and measurement related information to an information shell for each data file, which is accessed the "Information" icon (/) or <CTRL>+I (see section

4.4.5, page 92). With "Custom info" you can add further information about the sample and measurement.

3.5.1.1. Adding Information to the Custom Info Window

Open the "Custom info" dialog window by clicking on Acquisition > Custom info.



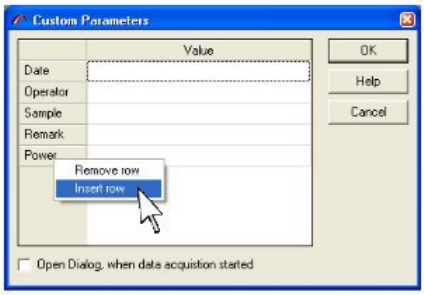
When the "Custom info" window is first opened it will show the default categories for "Date", "Operator", "Sample", "Remark" and "Power".

Left click in the desired "value" box and insert the relevant text. Add information for each category as required.

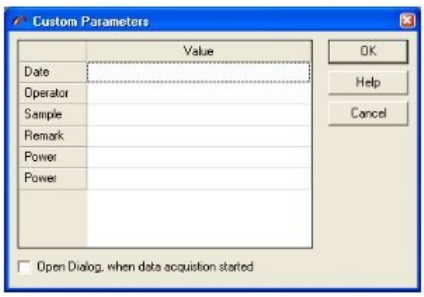
Tick the "Open Dialog, when data acquisition started" box if you want the "Custom info" window to automatically open at the start of each measurement. This can be useful to prevent you forgetting to add custom information to each data file.

3.5.1.2. Adding New Categories to the Custom Info Window

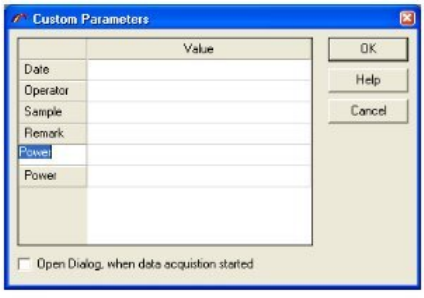
Additional categories can be added by right clicking on one of the category name boxes and selecting "Insert row".



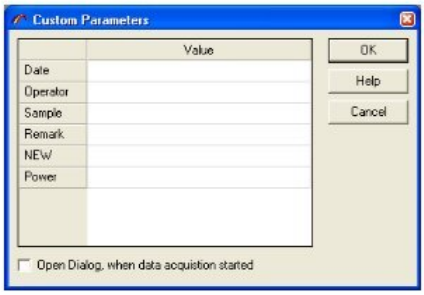
A new row will be inserted above the selected position, and will display the same name as the original category.



Double click on the category name box to allow the name to be edited. Type in the desired category name.

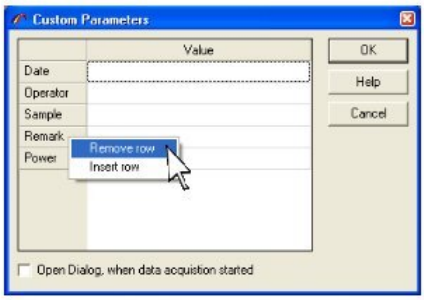


Click on any other category box to register the new name.

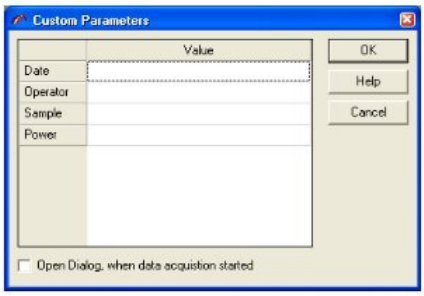


3.5.1.3. Deleting Categories from the Custom Info Window

Categories can be deleted by right clicking on the category name boxes which is to be deleted and selecting “Remove row”.



The category will be deleted from the Custom Info window.



3.5.2. Trigger

The “Trigger” dialog window allows you to configure manual trigger prompts which can be set to appear before an acquisition. Manual triggers are useful if you wish to start a measurement at a specific point in time, or if you wish to acquire an array of data manually. Once the trigger prompt **[OK]** is clicked then the measurement will start.

The window shows the trigger prompt message which will be displayed for a “Start” trigger (see section 3.5.2.1, page 33), and a “Sample” trigger (see section 3.5.2.2, page 33). If the “Use” box is ticked, then the trigger will be active, and a prompt window will be displayed before the acquisition.

3.5.2.1. Trigger "Start"

The trigger "start" message will be displayed at the start of a complete acquisition. In this case, a complete acquisition means acquisition of a single spectrum (/ ; see section 4.5.3, page 96) or multidimensional spectral array (/ ; see section 4.5.4, page 96) such as a profile (e.g., depth, time, temperature, time) or map (XYZ).

Click **[OK]** to start the measurement, or **[Cancel]** to abort the measurement.

3.5.2.2. Trigger "Sample"

The trigger "sample" message will be displayed at each measurement point in a spectrum array acquisition, such as a profile (e.g., depth, line, temperature, time) or map (e.g., XYZ). In addition, the "sample" message will be displayed before each window acquisition in an extended range measurement.

This function can be useful to allow manual focussing before each measurement point in an array acquisition.

Click **[OK]** to start the measurement, or **[Cancel]** to abort the measurement.

3.5.3. RTD

The "Real Time Display, RTD" dialog window allows you to control the two dimensional real time read out configuration of the CCD array detector – it is typically only used for diagnostic and maintenance purposes. Real time display of the CCD detector is activated using the / icon.

Image binning X
Select an appropriate binning factor from the drop down box for the X dimension of the CCD image.

Image binning Y
Select an appropriate binning factor from the drop down box for the Y dimension of the CCD image.

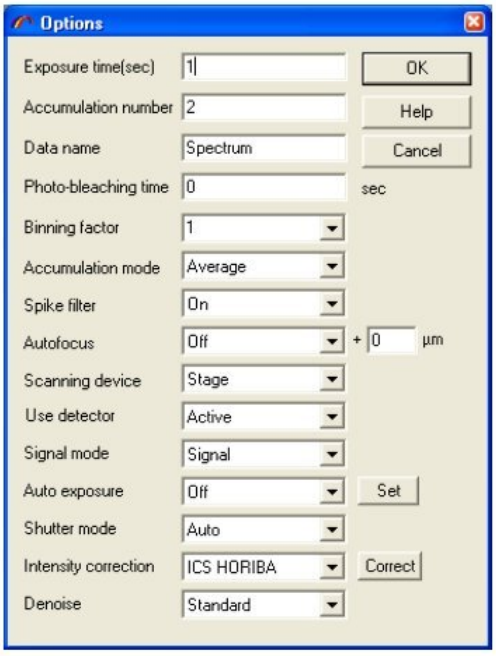
Readout zone
Select "Full" to read out the entire CCD array, or select "Spectrum" or "Image" to read out from the CCD zone used for spectrum or image acquisition. The "Spectrum" and "Image" read out zones are set in Acquisition > Detector – see section 3.5.7, page 57.

Accumulate image
Tick the box if you want sequential images from the CCD to be co-added to improve image quality and signal to noise ratio.

Exposure time (sec)
Set the default integration time to be used for the CCD real time display.

3.5.4. Options

The "Options" dialog window allows you to control a number of default settings which will be applied automatically for data acquisition. Most parameters controlled in Acquisition > Options are not changed for every acquisition, but rather are set according to a user's specific preferences and then applied to all acquisitions. Certain items which are required to be modified for each individual acquisition (i.e., "Exposure time", "Accumulation number" or "Data name") can be quickly accessed through the Control Panel (see section 9, page 227).



3.5.4.1. Exposure time (sec)

This sets the exposure time (or acquisition time) in seconds for an individual spectrum acquisition. The value displayed and set here is identical to that displayed and set in the Control Panel – see section 9.9.2, page 235.

3.5.4.2. Accumulation number

This sets the number of accumulations which allows multiple spectra to be averaged to improve spectrum quality and signal to noise ratio. The value displayed and set here is identical to that displayed and set in the Control Panel – see section 9.9.3, page 235.

3.5.4.3. Data name

This sets the data name tag, which is a prefix which is used for every newly acquired spectrum. In addition, it is used as the default file name during a File > Save As procedure. The value displayed and set here is identical to that displayed and set in the Control Panel – see section 9.8, page 234.

3.5.4.4. Photo-bleaching time

Photo-bleaching is a process whereby high fluorescence backgrounds are reduced by interaction of the sample with the high intensity laser beam used for Raman analysis. Photo-bleaching can assist in reducing backgrounds in a spectrum, and allowing a better quality Raman spectrum to be acquired.

When a photo-bleaching time is set in Acquisition > Options, once a measurement is started the software will wait for the specified photo-bleaching time before acquiring data (according to the specified acquisition time and number of accumulations – see section 9.9, page 234). This option is useful to have a controlled and consistent photo-bleaching time for a number of spectra.

When using the photo-bleaching function it is advisable to check that the sample behaviour is suitable for this function. Photo-bleaching depends on the fluorescence background reducing, but otherwise the sample must remain unchanged and chemically viable. In some cases, a reduction in fluorescence background may be accompanied by laser-induced burning of the sample, which is apparent through a changing Raman spectrum and/or visible of signs of burning on the sample surface. If this happens, then photo-bleaching cannot be used, and it is necessary first to reduce the laser power to prevent sample burning – this can be done by inserting a neutral density filter into the laser path (see section 9.2, page 227).

The photo-bleaching 'wait' time will be applied before the start of a single acquisition. Note that it will not be applied for the real time display (RTD) acquisition.

- For an extended range acquisition (see section 3.5.6, page 49) the photo-bleaching 'wait' time is applied before the first spectral window only.
- For a multidimensional spectral array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) the photo-bleaching 'wait' time is applied before each spectrum acquisition in the array.

When the photo-bleaching function is active (i.e., photo-bleaching time is >0s), an indicator will be displayed in the Status Bar.

3.5.4.4.1. Turning On the Photo-bleaching Function

In Acquisition > Options set the "Photo-bleaching time" to be any value greater than 0s.

Click on **[OK]**. The photo-bleaching function is now active.

The indicator icon is displayed in the Status Bar. 

3.5.4.4.2. Turning Off the Photo-bleaching Function

In Acquisition > Options set the "Photo-bleaching time" to be 0s.

Click on **[OK]**. The photo-bleaching function is now inactive.

The indicator icon is no longer displayed in the Status Bar.

3.5.4.5. Binning Factor

The "Binning Factor" controls the number of data points in a spectrum.

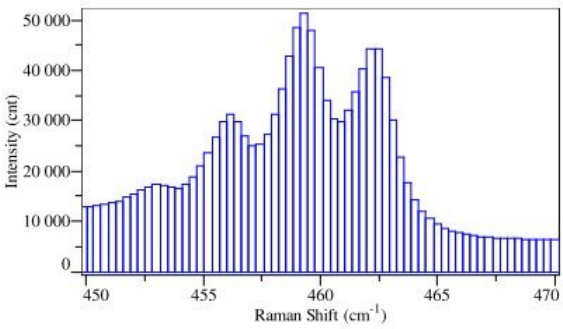
Binning Factor = 1 (default value)
When the "Binning Factor" is set to 1, the full number of data points is used to create the spectrum. Typically this will result in each spectral window having between 1000-1600 data points; the exact number depends on the specific configuration and set up of the Raman system.

The default value for "Binning Factor" is 1.

Binning Factor > 1
When the "Binning Factor" is set to >1, the number of data points used to create a spectrum is reduced, by co-adding adjacent pixels on the detector. Increasing the "Binning Factor" results in spectra which have less data points, and result in smaller file sizes; in addition, the spectral quality (e.g., signal to noise ratio) will be increased. However, spectral resolution will be correspondingly reduced, which may result in detailed spectral features being lost.

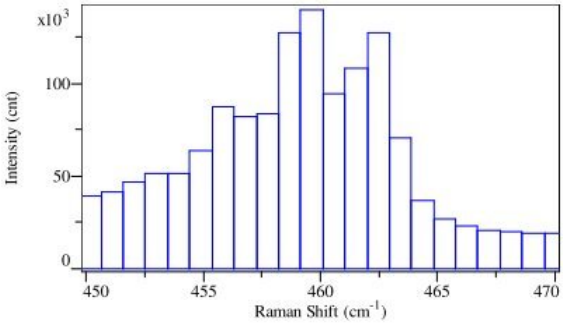
Typically using a "Binning Factor" > 1 is only suitable for specialized applications. If in doubt, use the default value of 1.

The data shown right has been acquired with "Binning Factor" = 1.



The data shown right is the same spectrum but acquired with "Binning Factor" = 3.

Note how there are 3x less data points, but the maximum signal level is 3x higher, resulting in a spectrum with increased spectral quality (e.g., signal to noise ratio). However, the detail of the spectrum (such as peak shape) is reduced.



3.5.4.6. Accumulation Mode

The "Accumulation Mode" sets how multiple accumulations (see section 9.9.3, page 235) are treated during an acquisition.

Average

Select "Average" from the drop down box to have multiple accumulations averaged together as they are displayed. The averaging is performed post-acquisition, after data transfer from the detector to the PC.

During an acquisition of multiple accumulations, the spectrum displayed on-screen represents the sequentially averaged data. For example, if three accumulations are specified the data will be displayed sequentially:

- Display 1: accumulation 1
- Display 2: Average(accumulation1 + accumulation2)
- Display 3: Average(accumulation1 + accumulation2 + accumulation3)

Sum

Select "Sum" from the drop down box to have multiple accumulations summed together before they are displayed. The summing is performed post-acquisition, after data transfer from the detector to the PC.

During an acquisition of multiple accumulations, the spectrum displayed on-screen represents the sequentially summed data. For example, if three accumulations are specified the data will be displayed sequentially:

- Display 1: accumulation 1
- Display 2: Sum(accumulation1 + accumulation2)
- Display 3: Sum(accumulation1 + accumulation2 + accumulation3)

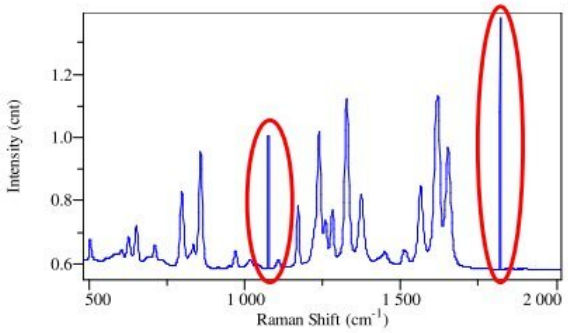
Detector

Select "Detector" from the drop down box to accumulate spectra on the detector prior to data transfer to the PC. This mode is only available for specific detectors.

3.5.4.7. Spike Filter

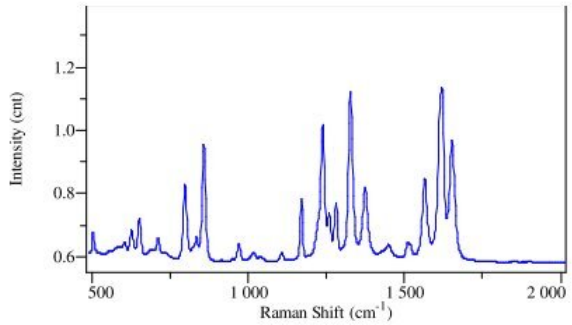
The "Spike Filter" is a sophisticated algorithm designed to remove random spikes (also known as cosmic rays) which can appear in any spectrum. These spikes are entirely natural, and do not indicate a fault with your system. Without the "Spike Filter" it is difficult to know whether an observed peak is real (e.g., Raman) or a spike artefact. With the "Spike Filter" active you are assured that all peaks in your spectrum are real.

The spectrum shown to the right was acquired without the "Spike Filter". Spikes in the spectrum are highlighted.



The "Spike Filter" algorithm compares multiple accumulations of a spectrum and calculates what peaks are real (e.g., Raman peaks) and what peaks are spikes. It then automatically removes spikes from the spectrum. The comparison method ensures that real peaks can never be incorrectly deleted.

The spectrum shown to the right was acquired with the "Spike Filter" active. This time all peaks in the spectrum are Raman peaks, and there are no spikes visible.



3.5.4.7.1. Turning On the "Spike Filter"

In Acquisition > Options select either "On (auto add)" or "On" from the "Spike Filter" drop down box.

- **On (auto add):** this mode automatically adds an extra accumulation to your measurement. For example, if you specify three accumulations in the control panel (see section 9.9.3, page 235) the actual number acquired will be four (3+1). This mode ensures that *every* spectrum will be spike filtered, even if you only specify one accumulation (since the software will automatically acquire two accumulations (1+1) to enable the "Spike Filter" comparison algorithm).
- **On:** this mode will activate the "Spike Filter" only if the number of accumulations set in the control panel (see section 9.9.3, page 235) is greater than 1. So, if an acquisition is made with 2,3,4... accumulations, then the "Spike Filter" will be active. If an acquisition is made with only 1 accumulation, then the "Spike Filter" will be inactive, and there is the possibility of spikes being present in your spectrum. This mode is useful since it allows you to easily switch between a fast analysis mode without the "Spike Filter" (e.g., when the accumulation number is set to 1), and standard analysis with the "Spike Filter" (e.g., when the accumulation number is set to 2 or more)

3.5.4.7.2. Turning Off the "Spike Filter"

In Acquisition > Options select "Off" from the "Spike Filter" drop down box.

3.5.4.8. Autofocus

The "Autofocus" function sets how the autofocus operation (see section 3.5.8, page 59) is applied to a measurement. There are three main modes of operation:

Before acquisition
In this mode, the autofocus procedure will be applied a single time at the start of a spectrum or array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes).

For example, if an XY map comprising 10 x 10 data points is to be acquired, the autofocus procedure will be applied once at the beginning to find the optimal focus point, and the 100 data points will then be acquired at the same optimal focus point.

This mode is useful when you wish to use autofocus for single spectrum acquisition, or for surface mapping of a flat, smooth sample.

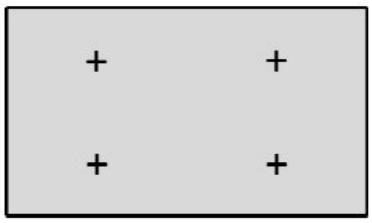
Before each point
In this mode, the autofocus procedure will be applied for each individual spectrum being measured in the acquisition. For an array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) this means that the autofocus will be performed before each point in the array.

For example, if an XY map comprising 10 x 10 data points is to be acquired, the autofocus procedure will be applied at each XY coordinate prior to spectrum acquisition.

This mode is useful when you wish to use autofocus for mapping of a rough and/or non-flat (tilted) sample. If the sample is smooth but non-flat (tilted) then the "In limits point" mode is recommended – see below.

In limits point
In this mode, the autofocus procedure will be applied at four intermediate positions in a rectangular surface (XY) mapping area to calculate the tilt of the sample. The focus position will then be adjusted at each measurement point to compensate for the tilt.

In the diagram below showing a surface (XY) mapping area the intermediate "In-limits point" autofocus points are marked with with "+".



For example, if an XY map comprising 10 x 10 data points is to be acquired, the autofocus procedure will be applied at the intermediate positions of the map area prior to the mapping. When the map acquisition starts, the focus position will be adjusted at each point, based on the tilt plane calculated by the autofocus.

This mode is useful when you wish to use autofocus for mapping of a smooth but non-flat (tilted) sample.

3.5.4.8.1. Turning On Autofocus

In Acquisition > Options select either "Before acquisition", "Before each point" or "In limits point" from the "Autofocus" drop down box.

Click **[OK]**. Autofocus is now active.

The Autofocus indicator icon is displayed in the Status Bar. 

3.5.4.8.2. Setting the Autofocus Offset

Depending on the exact Autofocus mode being used (see section 3.5.8, page 59) the focus position located by the autofocus procedure may correspond to a visual focus of the sample, which is not always the position giving a maximum Raman signal.

In this case, it is possible to set a Z-axis offset, which will be applied after the autofocus position has been located. A negative offset means that the focus will be moved upwards (e.g., analysis will be made higher in the sample than the autofocus position); a positive offset means that the focus will be moved downwards (e.g., analysis will be made lower in the sample than the autofocus position).

The offset is also useful if you wish to do mapping inside a sample at a fixed position below the surface. The autofocus procedure (depending on the exact mode being used – see section 3.5.8, page 59) will locate the surface of the sample, and the positive offset will then be applied to move to a specified position below the surface.

3.5.4.8.3. Turning Off Autofocus

In Acquisition > Options select "Off" from the "Autofocus" drop down box.

Click **[OK]**. Autofocus is now inactive.

The Autofocus indicator icon is no longer displayed in the Status Bar.

3.5.4.9. Scanning Device

The "Scanning Device" function sets what device is used for Y axis movement in multidimensional spectral array measurements (such as XY surface maps, or YZ depth slices).

The default mode is "Stage" which uses the motorized sample XY stage to move the sample in both X and Y dimensions.

On systems which are equipped with the confocal LineScan mirror scanning hardware it is also possible to use the LineScan to acquire data in the Y axis. In this case select "Scanner" to disable the XY motorized stage for Y axis movement during the measurement, and to use the LineScan mirror.

3.5.4.10. Use Detector

The "Use detector" function sets how multiple detectors are used during a measurement. If your Raman system is equipped with a single detector only, then this option will be set to "Active" and will be greyed out.

3.5.4.10.1. Setting the Use Detector Mode

In Acquisition > Options select either "Active" or "Both" from the "Use Detector" drop down box.

Active
In this mode data will only be acquired from the active detector. The active detector is selected from the status bar (see section 7.3.2, page 201).

Both
In this mode it is possible to acquire data from two detectors simultaneously, but this only applies to very specific configurations. For most standard systems, it is only ever possible to acquire data from the active detector.

3.5.4.11. Signal Mode

The "Signal Mode" function controls whether a 'dark' subtract procedure is implemented automatically during a spectrum acquisition.

Some detectors (e.g., the InGaAs near infra-red array detector) have a significant fixed pattern artefact signal which can dominate the spectrum acquired from a sample with low signal level. In this case, it is possible to acquire a 'dark' spectrum (e.g., a spectrum acquired with a shutter in front of the detector, so that no sample signal is observed) which corresponds to the fixed pattern signal only. By performing a 'dark' subtract (e.g., subtract the 'dark' spectrum from the sample spectrum) significant improvement in spectrum quality can be realised.

3.5.4.11.1. Setting the Signal Mode

In Acquisition > Options select either "Signal", "Dark", "Signal-Dark" or "Signal-Dark (always)" from the "Signal Mode" drop down box. These choices what type of spectrum is acquired from the detector.

Signal
In this mode, the sample spectrum (detector shutter open) will be acquired and displayed on screen.

Dark
In this mode, the 'dark' spectrum (detector shutter closed) will be acquired and displayed on screen. Note that in this mode you will not see a spectrum corresponding to your sample.

Signal-Dark
In this mode, the 'dark' spectrum (detector shutter closed) will be automatically subtracted from the sample spectrum (detector shutter open). The 'dark' spectrum will be acquired first, followed by the sample spectrum.

The 'dark' spectrum will be acquired with identical acquisition settings as the sample spectrum – for example, if the acquisition is set for two accumulations of 30s each, then the 'dark' spectrum will also

be acquired with two accumulations of 30s each. As a result, the total acquisition time will be doubled.

In the case of an extended range spectrum acquisition the 'dark' spectrum will be acquired once at the start of the extended range acquisition only, and then subtracted from each spectral window. In the case of an array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes), the 'dark' spectrum will be acquired once at the start of the acquisition only, and then subtracted from the spectrum acquired at each measurement point.

This mode is useful when the 'dark' spectrum is constant, and is not expected to change between measurements.

Signal-Dark (always)
This mode is similar to "Signal-Dark" discussed above. However, in this case, the 'dark' spectrum is acquired before each and every readout from the detector.

In the case of an extended range spectrum acquisition the 'dark' spectrum will be acquired for each spectral window and subtracted from the sample spectrum. In the case of an array acquisition (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes), the 'dark' spectrum will be acquired and subtracted at each measurement point.

This mode is useful when the 'dark' spectrum is expected to vary between measurements.

3.5.4.12. Autoexposure

The Auto Exposure function sets whether the auto exposure operation (see section 3.5.10, page 65) is applied to a measurement.

3.5.4.12.1. Turning Auto Exposure On

In Acquisition > Options select "On" from the "Autoexposure" drop down box.

When Auto Exposure is active, the Acquisition Time section in the Control Panel is set to "Auto" and greyed out.

3.5.4.12.2. Turning Auto Exposure Off

In Acquisition > Options select "Off" from the "Autoexposure" drop down box.

3.5.4.12.3. Setting Up the Auto Exposure

In Acquisition > Options click on **[Set]** in the "Autoexposure" section. See section 3.5.10, page 65, to learn how the Auto Exposure function can be configured.

3.5.4.13. Shutter Mode

The "Shutter Mode" function sets the operation of the detector shutter during an acquisition.

3.5.4.13.1. Setting the Shutter Mode

In Acquisition > Options select "Auto", "Open" or "Closed" from the "Shutter Mode" drop down box.

Auto
In this mode, the shutter operation is set automatically by LabSpec 5. Typically this means that for standard spectrum and array acquisition the shutter will be opened during spectrum acquisition, and closed during detector readout and other system processes (such as moving the sample stage, or spectrometer grating). For ultra-fast SWIFT™ mapping (see 4.5.5, page 97) the shutter is opened at the start of the acquisition, and then kept open throughout the acquisition. It is closed only once the final spectrum has been acquired.

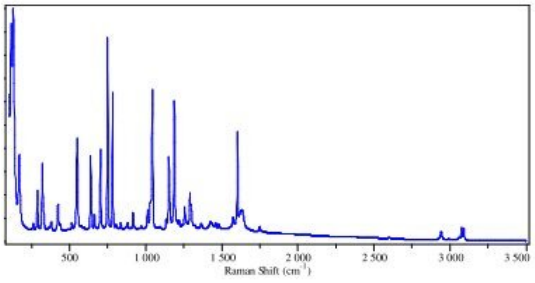
Open
In this mode, the shutter will be kept open throughout an acquisition. For example, for an extended range spectrum acquisition (see section 3.5.6, page 49) the shutter will be opened prior to acquisition of the first spectral window, and will be kept open throughout the acquisition. It is closed only once the final spectral window has been acquired.

Closed
In this mode, the shutter will be kept closed throughout an acquisition – it can be used to monitor the inherent background 'dark' response of the detector. This mode can be used to assess the spectrum of a detector without any light. Note that in this mode you will not see a spectrum corresponding to your sample.

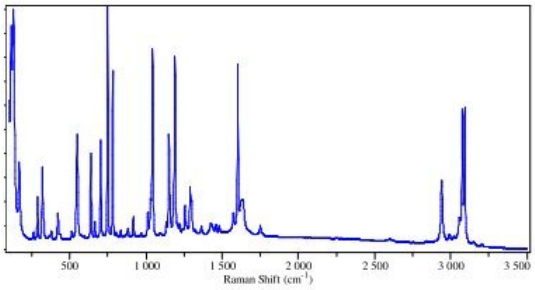
3.5.4.14. Intensity Correction

The LabSpec 5 intensity correction algorithm allows true comparison of spectra acquired using different laser wavelengths and optics. Without intensity correction, spectra acquired using different laser wavelengths and optics can show significant differences in relative peak intensities. These differences are caused by varying performance of the instrument components (including microscope objectives, laser rejection filters, diffraction gratings and CCD detectors) at different wavelengths.

The spectrum shown right for 4-acetyl salicylic acid was acquired using a 785 nm near infra-red laser. In this case the CCD detector sensitivity decreases as the Raman shift increases. Thus peak intensities at high Raman shift positions are observed to be significantly weaker than they really are. The observed spectrum is not correct, since it is perturbed by instrumental factors.



By applying the "HORIBA ICS" intensity correction factor the perturbed Raman spectrum peak intensities can be restored, as illustrated in the corrected spectra shown right.



3.5.4.14.1. How is the "HORIBA ICS" Correction Factor Calculated?

The "HORIBA ICS" intensity correction factor is created individually for each instrument at the time of manufacture.

The output from a calibrated white light source is passed through the system, and the recorded "response" spectrum is compared with the calibrated "source" spectrum of the white light source. The correction factor is calculated as

correction = [*source*] / [*response*]

The obtained correction factor is a multiplicative factor, which means that each spectrum acquired is multiplied by the correction factor to yield the corrected spectrum.

A correction factor is created for each and every pairing of laser wavelength and diffraction grating available on the instrument (e.g., 532nm-600gr/mm; 532nm-1800gr/mm; 633nm-600gr/mm; 633nm-1800gr/mm etc). When the automatic intensity correction is activated, the software automatically applies the appropriate correction factor based on the active laser wavelength and diffraction grating pair.

In the event that there are multiple laser rejection filters for a single laser wavelength configured on a LabRAM HR system (e.g., one notch filter and one edge filter for 633nm) the software cannot automatically detect which filter is in place. In this case, the specific laser filter must be selected from Setup > ICS Filters before the acquisition is made – see section 3.7.3, page 75, for further information.

3.5.4.14.2. Turning On the Automatic Intensity Correction

In Acquisition > Options, select "HORIBA ICS" from the "Intensity Correction" drop down box.

Click **[OK]**. Intensity Correction is now active, and will be applied to all acquired data.

The Intensity Correction indicator icon is displayed in the Status Bar.

If multiple injection/rejection or photoluminescence (PL) filters have been set up for a single laser wavelength ensure the correct filter is selected

from the ICS Filters dialog window, by clicking on Setup > ICS Filters.

Note that an intensity correction factor must have been created for the specified laser wavelength and diffraction grating pair, otherwise an error message similar to that shown right will be displayed when an acquisition is started. Click on **[OK]** or **[Cancel]** to clear the message, and start the acquisition. Remember that in this case the spectrum will be uncorrected. The error message will only be shown once during a LabSpec session – if LabSpec is closed and then re-opened, the message will be displayed again if intensity correction is activated when no intensity correction factor has been created.

3.5.4.14.3. Turning Off the Automatic Intensity Correction

In Acquisition > Options, select "Off" from the "Intensity Correction" drop down box.

Click **[OK]**. Intensity Correction is now inactive.

The Intensity Correction indicator icon is no longer displayed in the Status Bar.

3.5.4.14.4. Applying Post-acquisition Intensity Correction

Ensure that the correct laser wavelength and diffraction grating pair are selected in the Control Panel.

If multiple injection/rejection or photoluminescence (PL) filters have been set up for a single laser wavelength ensure the correct filter is selected from the ICS Filters dialog window, by clicking on Setup > ICS Filters.

Select the spectrum which is to have the Intensity Correction applied post-acquisition.

In Acquisition > Options, select "HORIBA ICS" from the "Intensity Correction" drop down box.

Click on **[Correct]** in the Intensity Correction section.

Click **[OK]**. The Intensity Correction has now been applied to the spectrum.

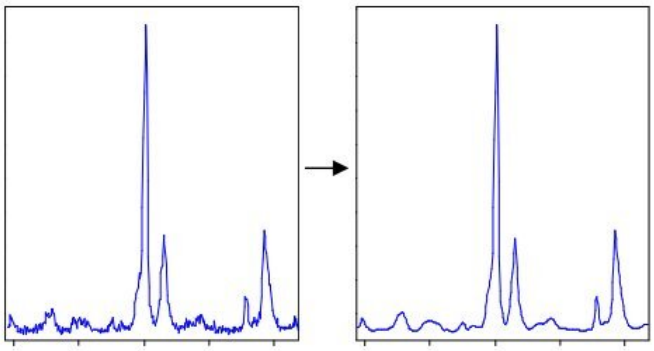
Note that an intensity correction factor must have been created for the specified laser wavelength and diffraction grating pair, otherwise an error message similar to that shown right will be displayed when an acquisition is started. Click on **[OK]** or **[Cancel]** to clear the message. Remember that in this case the spectrum will remain uncorrected. The error message will only be shown once during a LabSpec session – if LabSpec is closed and then re-opened, the message will be displayed again if intensity correction is activated when no intensity correction factor has been created.

3.5.4.15. Denoise

The Denoise function is a unique noise reduction algorithm which can be used to significantly enhance spectrum quality without losing subtle spectral information. When Denoise is activated in Acquisition > Options, all data acquired will be processed with the Denoise reduction – this includes real time display (RTD) acquisition (/), spectrum acquisition (/) and multidimensional spectral array acquisition (/).

Standard smoothing functions can result in loss of peak shape and position, and subtle features (such as weak shoulders on a strong band) can be lost. The Denoise function ensures that all this important information is retained, whilst still reducing noise in the spectrum.

The spectra shown right illustrate the effect of the Denoise function.



Two main Denoise algorithms are available from the "Denoise" drop down box:

- Standard: recommended for spectra with signal to noise ≥ 20
- Lite: recommended for very noisy spectra with signal to noise ≤ 20

In addition, both algorithms can be used with an integrated Despike function to remove random spikes (also known as cosmic rays). See also section 3.5.4.7, page 37, for more information about other spike filter options in LabSpec 5.

Denoise can be applied post-acquisition using the Smoothing icon and Filtration dialog window – see section 4.6.4, page 124. If a spectrum has had the Denoise function automatically applied through Acquisition > Options, it cannot have the function applied again through the Filtration dialog window.

3.5.4.15.1. Turning On the Automatic Denoise Function

In Acquisition > Options select the desired Denoise function from the "Denoise" drop down box:

- Standard: recommended for spectra with signal to noise ≥ 20
- Standard + Despike: as "Standard" but with an integrated Despike function to remove random spikes (also known as cosmic rays)
- Lite: recommended for very noisy spectra with signal to noise ≤ 20
- Lite + Despike: as "Lite" but with an integrated Despike function to remove random spikes (also known as cosmic rays)

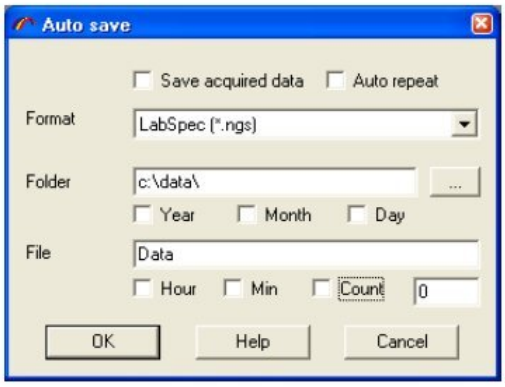
Note that this function will be automatically applied to all data acquisition.

3.5.4.15.2. Turning Off the Automatic Denoise Function

In Acquisition > Options select "Off" from the "Denoise" drop down box.

3.5.5. Auto save

The autosave function allows data to be saved automatically when a measurement is completed. This function is useful to ensure that important data is immediately saved, so that there is no risk of loss of data caused by intervention of another user, power cut, or computer crash.



Note that the autosave function can only be activated for spectrum acquisition (/) and multidimensional spectral array acquisition (/). Real time display (RTD) data (see section 4.5.1, page 95) will not be autosaved, even if the autosave function is activated in LabSpec 5.

3.5.5.1. Setting the Autosave Function

The Autosave function can be configured to automatically save data in a specific format to a specific location.

Save acquired data
If the "Save acquired data" box is ticked the Autosave function is active, and data will be automatically saved.

Auto repeat

If the "Auto repeat" box is ticked a spectrum acquisition (/) will be continuously repeated until the STOP icon (/) is clicked. Each spectrum displayed on the screen will be replaced by the next spectrum.

If the "Save acquired data" box is also ticked each individual spectrum will be automatically saved.

Format

Select the file format from the drop down box which will be used to save the data. Please see section 3.1.1.1, page 10, for full details about available file formats.

- LabSpec (*.ngs) – data will be saved in its inherent LabSpec 5 format. Spectra will be saved as .ngs, and multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) will be saved as .ngc.
- Dilor (*.ms0) – data will be saved a legacy file format for Dilor Raman instruments.
- Grams (*.spc) – data will be saved in a Grams SpectraCalc spectrum file format, compatible with many common spectroscopy software packages.
- LabSpec (*.tsf) – data will be saved in the previous generation LabSpec 4 format. Spectra will be saved as .tsf, and multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) will be saved as .tvf.

Folder

Type in the file path (or browse using the file browse button) for the location where the data should be saved.

- If the folder exists already, data will be saved inside the specified folder. Tick the boxes for "Year", "Month" and "Day" to create a new folder labelled "_YYYY_MM_DD", which indicates the year (YYYY), month (MM) and day (DD) according to the computer's clock.
- If the folder does not already exist it will be created when the Autosave procedure is next performed. If the boxes for "Year", "Month" and "Day" are ticked, the year (YYYY), month (MM) and day (DD) according to the computer's clock will be appended to the specified folder name (for example, "Folder_YYYY_MM_DD").

File

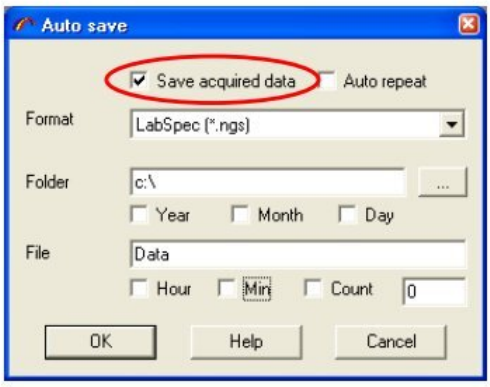
Type in the file name for the data which is to be saved. Tick the "Hour", "Min" and/or "Count" boxes to append the hour (HH), minute (mm) and/or integer count (C) to the file name. The count number will start at the number indicated in the box – to start the count at a different number type in the desired first number in the count box. This number will be automatically incremented for subsequent autosaves.

For example, with "Hour" and "Min" ticked, the data will be saved as "FileName_HH_mm.xxx" where ".xxx" indicates the specified file format.

With "Count" ticked, the data will be saved as "FileName_C.xxx" where ".xxx" indicates the specified file format.

3.5.5.2. Turning On the Autosave Function

In Acquisition > Autosave, tick the box for "Save acquired data".

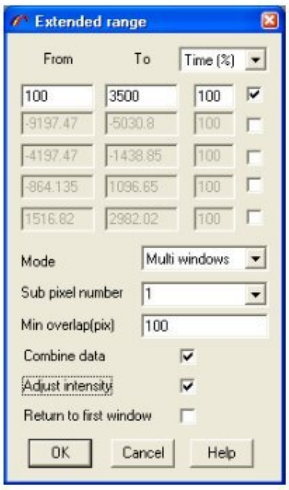


3.5.5.3. Turning Off the Autosave Function

In Acquisition > Autosave, untick the box for "Save acquired data".

3.5.6. Extended Range

The Extended Range dialog window controls the spectral range which will be analysed during a spectrum acquisition (/) or multidimensional spectral array acquisition (/). When an extended range is specified, the software will acquire multiple spectra and seamlessly glue them to give a final spectrum corresponding to the desired extended range.



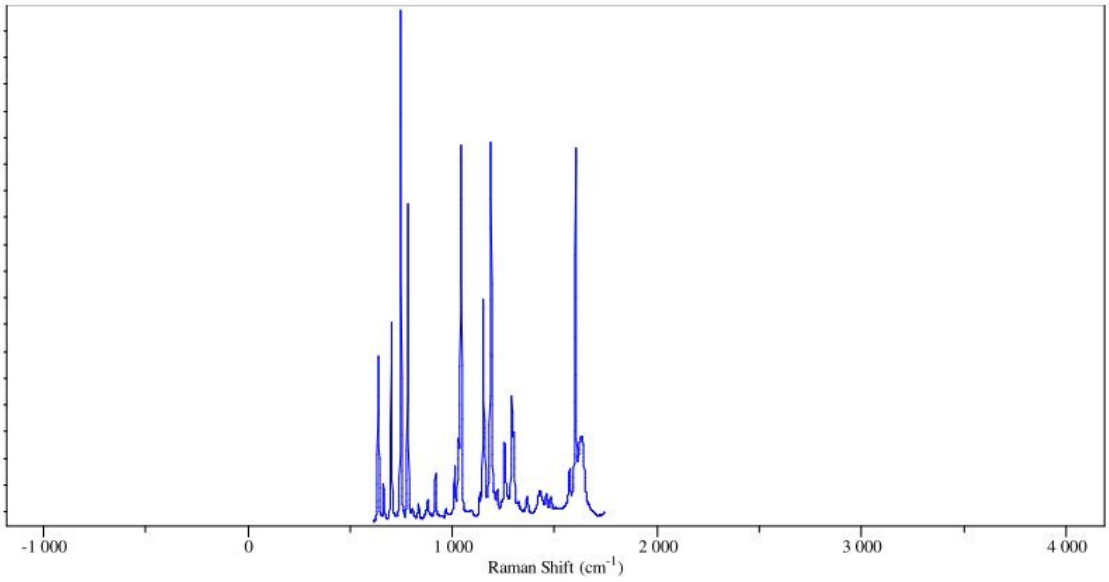
In the description that follows the term "Spectral Range" refers to the specified extended range, and "Spectral Window" refers to the individual spectra which are acquired to cover the extended range.

3.5.6.1. Extended Range Acquisition Modes

The Extended Range module offers three main modes for spectrum acquisition.

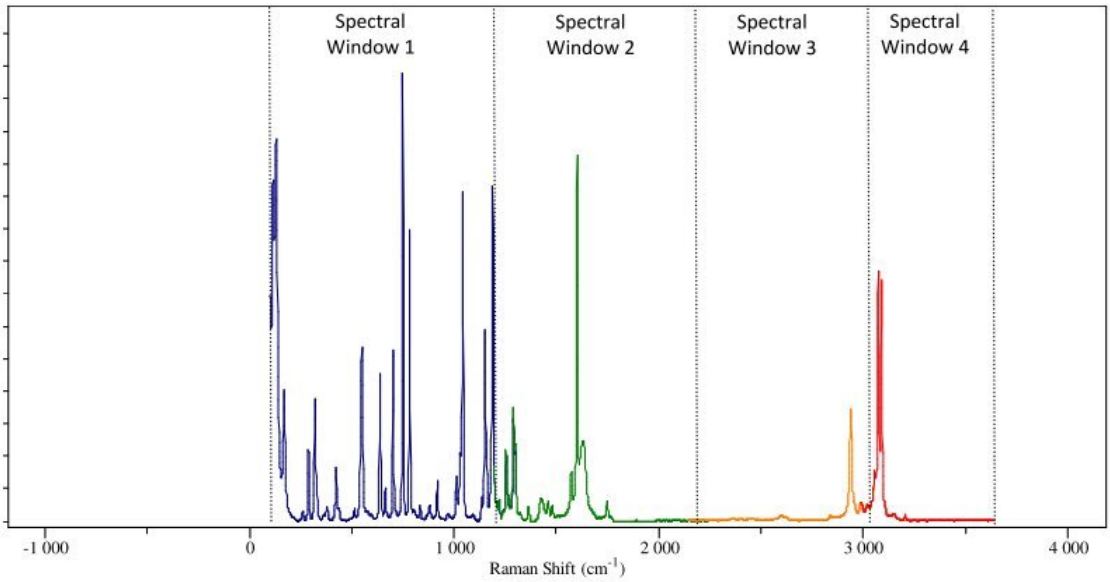
Single Window

In Single Window mode, only the spectral range covered by a single CCD detector readout will be used. The actual range will depend on the spectrometer position (see section 9.5, page 230), the laser wavelength (see section 9.1, page 227), and the diffraction grating (see section 9.6.1, page 231). Remember that the acquired spectrum will be centered about the current spectrometer position. In the example below, data has been acquired from 4-acetylsalicylic acid with the spectrometer centered at 1200cm^{-1} in Single Window mode.



Multiwindow

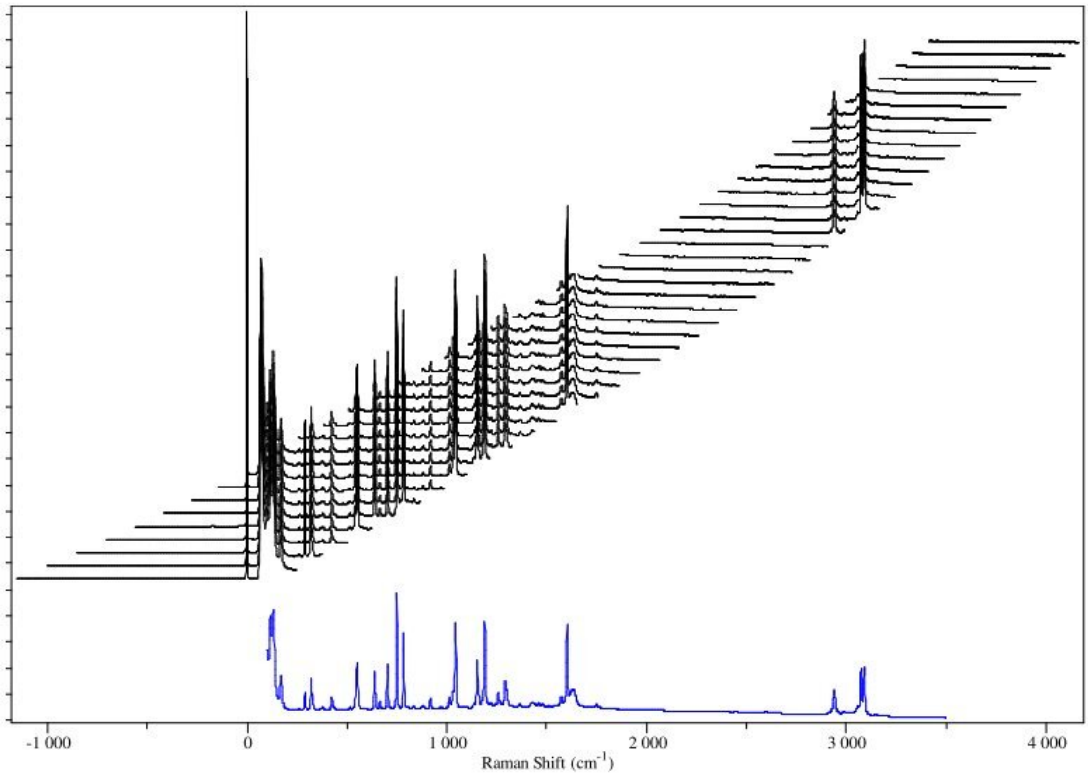
In Multiwindow mode, a user defined spectral range can be covered, with the software automatically adjusting the spectrometer position and capturing multiple spectral windows to cover the desired range. In the example below, data has been acquired from 4-acetylsalicylic acid in the range 100-3500cm^{-1} using the Multiwindow mode.



Auto scanning

In Auto scanning mode the spectrometer is moved in a number of very small steps, allowing the spectrum to be slowly built up step by step. The advantage of this method is that any pixel to pixel variation in response can be averaged out over the spectrum. In the example below, data has been acquired from 4-acetylsalicylic acid in the range 100-3500cm^{-1} using the Autoscanning mode.

The stacked spectra (—) show the individual spectra which are acquired, but they have been offset for clarity. The blue spectrum (—) shows the final spectrum as observed by the user, which is an average of all the individual spectra, in the range 100-3500cm^{-1}.



3.5.6.2. Sub-pixel Acquisition Function

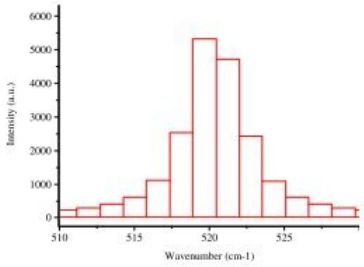
The Sub-pixel acquisition function uses the high precision, fine step size of the spectrometer drive mechanism to acquire spectra which are shifted by less than a whole pixel value. The result is to increase the number of data points defining a band (i.e., sub-pixel = 2 results in twice the number of data points; sub-pixel = 3 results in three times the number etc).

Note that this will increase the total acquisition time, and it is suggested that sub-pixel acquisition is only used over limited ranges.

The sub-pixel acquisition function does not increase the spectral resolution of the spectrometer, since this is defined principally by the laser wavelength, spectrometer focal length, diffraction grating and slit. However, the sub-pixel acquisition function is useful to give increased definition of a peak, by providing more data points to define that peak. This can be beneficial when studying peak shape, or performing peak fitting routines.

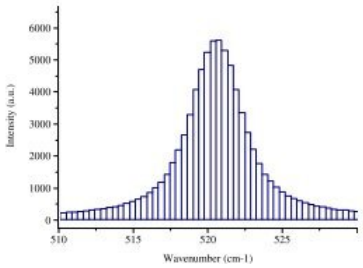
Sub-pixel = 1

The data shown right has been acquired with sub-pixel = 1, which is the default value for standard acquisitions.



Sub-pixel = 4

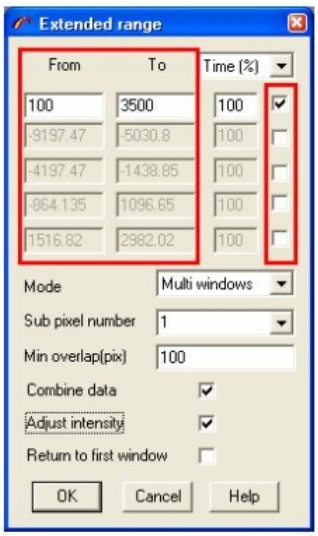
The data shown right has been acquired with sub-pixel = 4. The increased number of data points is clearly visible.



3.5.6.3. Setting the Extended Range

From and ***To***

Set the beginning ("From") and end ("To") spectral positions by typing in the desired values into the respective boxes. The spectral units are those which are displayed in the spectrometer window in the control panel (see section 9.5, page 230), and/or selected *via* Options > Unit (see section 3.4.1, page 22).



Note that multiple spectral windows can be set up by ticking the check boxes on the right hand side. Up to five spectral ranges can be defined. Each spectral range can be acquired with a specific integration time – see ***Time*** below.

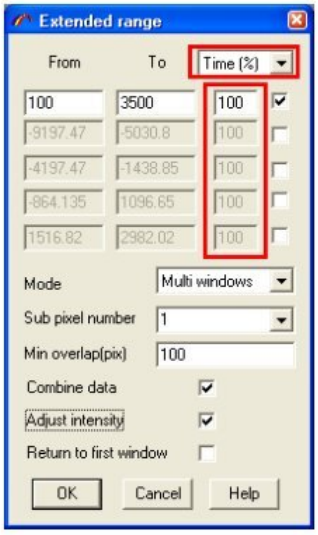
Multiple spectral ranges can be contiguous (e.g., 100-200, 200-500, 500-2000cm^{-1}) or discrete (e.g., 100-1500, 1750-1900, 2900-4000cm^{-1}).

For most standard measurements it is generally sufficient to use a single spectral range covering the whole range desired.

Time

The acquisition time for each spectral range can be set up in two ways.

- Time (%) – in this mode, the time in the Extended Range dialog window is specified as a percentage (%) of the acquisition time set in the Control Panel (see section 9.9.2, page 235). For example, if the Control Panel has the acquisition time set as 10s, then if Time (%) is set to 50% in the Extended Range dialog window, the acquisition will be made with 5s per spectral window.



- Time (s) – in this mode, the time in the Extended Range dialog window is specified directly in seconds (s). In this case the time set in the Control Panel (see section 9.9.2, page 235) is ignored.

Mode

Select "Single Window", "Multiwindow" or "Auto scanning" from the "Mode" drop down box to choose the desired acquisition mode for the extended range measurement. See section 3.5.6.1, page 50, for a detailed explanation of these modes.

Note that if you use "Auto scanning" it is necessary to set a larger number of accumulations in the Control Panel (see section 9.9.3, page 235). Typical values for good results would be between 10 and 50. The larger the number of accumulations, the better the averaging will be, resulting in a higher spectrum quality (signal to noise).

Sub-pixel Number

Select the sub-pixel number from the drop down box to determine whether data acquisition will be made in the default manner (sub-pixel = 1), or with additional data points to enhance band definition (sub-pixel > 1). See section 3.5.6.2, page 52, for a detailed explanation of this function, and how it affects the acquired data.

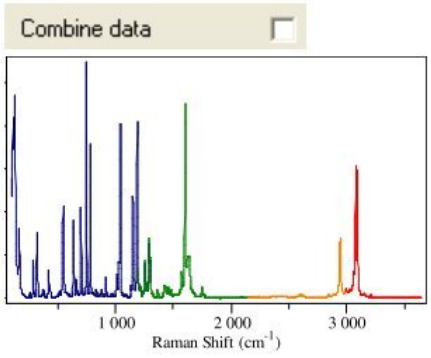
Min Overlap (pix)

In the Multiwindow acquisition mode each spectral window overlaps with its neighbours. The "Min Overlap (pix)" allows the user to define (in pixels) the minimum overlap which must be used. Typically a value of 100 is adequate for most measurements.

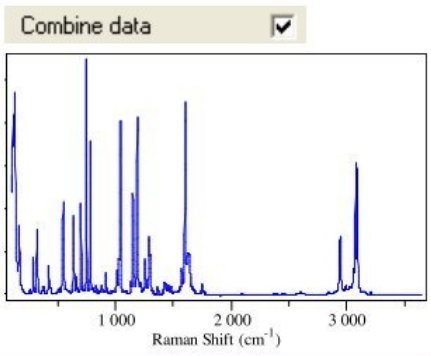
Combine Data

When "Combine Data" is ticked, the individual spectral windows will be automatically glued together to yield a single spectrum at the completion of the measurement.

Spectrum acquired with "Combine Data" unticked:



Spectrum acquired with "Combine Data" ticked:

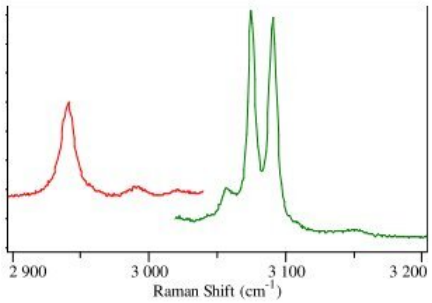


Adjust Intensity

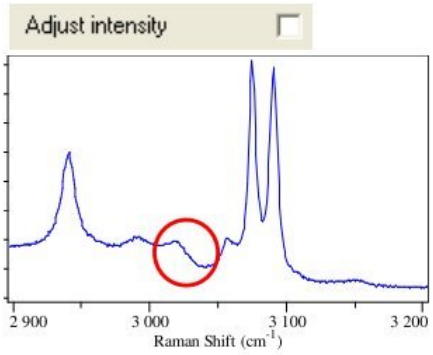
During an extended range measurement, discrete spectral windows are acquired to cover the entire range. As the spectrometer is moved to capture each spectral window there is a natural small drop in efficiency of the diffraction grating, which can result in slight changes to the baseline of each spectral window. When these spectral windows are glued to create the final spectrum covering the extended range these baseline changes can give rise to small steps in the spectrum.

If "Adjust Intensity" is ticked, the baselines of the individual spectral windows will be adjusted prior to gluing, to yield a seamless final spectrum.

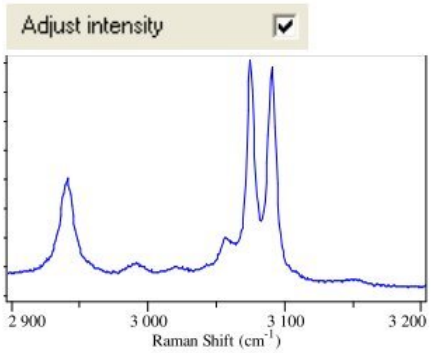
Raw data, showing the individual spectral windows with a natural baseline shift caused by changing efficiency of the spectrometer's diffraction grating.



Combined data, with "Adjust Intensity" unticked. Note the step at 3030 cm^{-1}.



Combined data, with "Adjust Intensity" ticked. The gluing between the two individual spectral windows is seamless.



Return to First Window

If "Return to First Window" is ticked, at the end of an extended range measurement the spectrometer will be returned to the starting position for the measurement, so that it is ready to immediately start the next measurement. If "Return to First Window" is unticked, the spectrometer will remain at the position used for the last spectral window. When a new measurement is started, then the spectrometer will move to the correct starting position for that measurement.

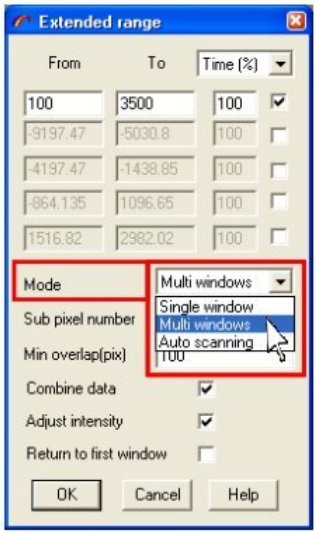
The "Return to First Window" function is particularly useful when making kinetic time based measurements with the extended range function. In this case it is important that the spectrometer is ready to immediately start the measurement at the next trigger point for the kinetic run, and thus, it must be at the starting position for the extended range in advance.

3.5.6.4. Turning On the Extended Range Function

In the extended range dialog window (accessed *via* Acquisition > Extended range, or the Extended range icon /) select the desired extended range mode from the "Mode" drop down box.

Choose either "Multi windows" or "Auto scanning" to make an extended range measurement.

Set up the spectral range and other options (section 3.5.6.3, page 53), and click **[OK]**. The extended range function is now active.

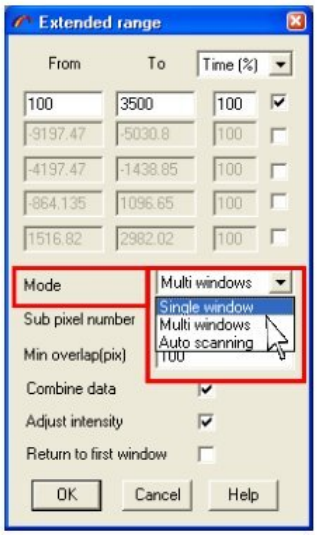


The extended range indicator icon is displayed in the Status Bar.

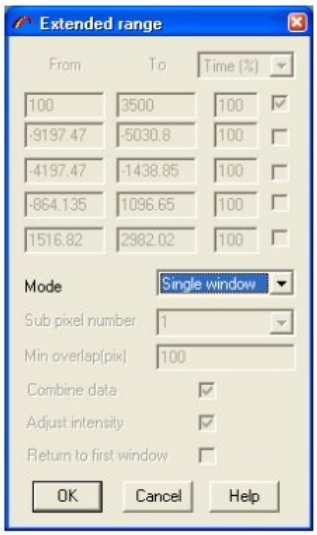
Note that the extended range is only available for full spectrum acquisition (/), or multidimensional spectral array acquisition (/). It is not available for real time display (RTD) (/).

3.5.6.5. Turning Off the Extended Range Function

In the extended range dialog window (accessed *via* Acquisition > Extended range, or the Extended range icon /) select the "Single window" mode from the "Mode" drop down box.



All the options will be greyed out.

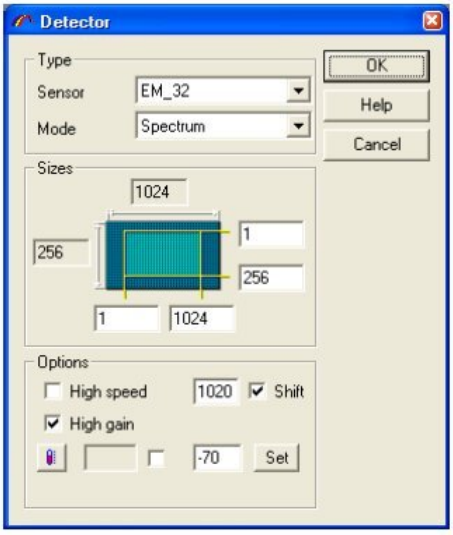


Click **[OK]**. The extended range function is now inactive.

The extended range indicator icon is no longer show in the Status Bar.

3.5.7. Detector

The detector window controls the configuration of the detector(s) used by the Raman system. In typical use these parameters do not need to be adjusted by the user, and it is recommended that they are only modified when instructed to do so by a trained engineer from HORIBA Scientific or one of its representatives.



CAUTION: CHANGING SETTINGS IN THE ACQUISITION > DETECTOR WINDOW MAY RESULT IN SYSTEM MALFUNCTION. ON NO ACCOUNT SHOULD SETTINGS IN THIS DIALOG WINDOW BE MODIFIED EXCEPT WHEN INSTRUCTED TO DO SO BY A TRAINED ENGINEER FROM HORIBA SCIENTIFIC OR ONE OF ITS REPRESENTATIVES.

Type

Select the detector to be used from the "Sensor" drop down box – this list will only show the detectors installed on the instrument.

Note that when multiple detectors are installed the active detector can also be selected using the detector indicator in the Status Bar (see section 7.3.2, page 201).

Select the read out mode for the detector from the "Mode" drop down box. For two dimensional array detectors (such as a CCD), the software has different read out zones (areas) configured, one for spectrum acquisition, and one for area intensity acquisition.

- Spectrum – select this mode for acquiring normal spectra where the detector is used in a one dimensional configuration (spectral axis).
- Image – select this mode for specialized applications where the detector is used in a two dimensional configuration (spectral axis and CCD height).

Sizes

The "Sizes" section controls what area of the detector is used for the "Spectrum" and "Image" modes. Type in the desired minimum and maximum pixel numbers to define the area which will be used. The values in the greyed out boxes indicate the maximum pixel size of the detector in the X and Y axes.

Options

The options visible will depend on the particular detector(s) installed on the instrument. Please consult a trained engineer from HORIBA Scientific or one of its representatives to find out more about the options available for the detectors installed on your system.

In most cases, the temperature sensor and control options will be present, and these are described below.

3.5.7.1. Displaying the Detector Temperature

Click on the "temperature" icon to display the current temperature of the detector.

The current temperature (in degrees celsius, °C) will be displayed in the adjacent indicator box.

Tick the box to have the current temperature continuously updated.

Note that the current temperature of the active detector is also displayed next to the detector indicator icon in the Status Bar.

3.5.7.2. Setting the Detector Temperature

To set the temperature of the detector, type in the desired value (in degrees celsius, °C), and click **[Set]**.

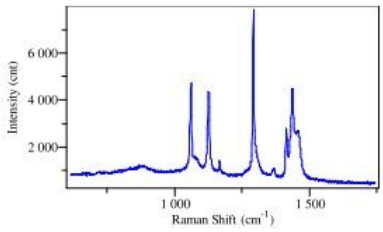
3.5.8. Autofocus

The Autofocus function in LabSpec 5 automatically finds the optimum focus position for a sample, and ensures that the best quality spectrum with maximum signal level can be easily achieved.

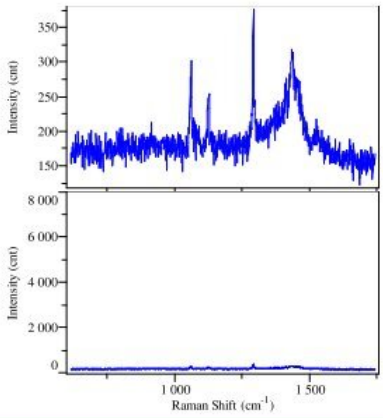
It is a useful tool for samples where manual focusing is difficult (such as highly polished, clean surfaces). So long as the sample is relatively close to focus the Autofocus procedure will find the correct focus position and give a good quality spectrum.

Autofocus also is invaluable when acquiring XY surface maps of samples which have significant surface roughness. Without autofocus whenever the measurement position in the map is out of focus the signal level will be reduced, and analysis of the map data will be difficult. With the autofocus running, the focus position will be adjusted at each measurement position in the map, ensuring that good quality data is acquired across the full mapping area, despite the sample's inherent roughness.

Spectrum acquired using 100x objective with autofocus.



Spectrum acquired using 100x objective without autofocus; out of focus by approximately 20 µm. The bottom image is shown with the same intensity scale as the 'autofocus' spectrum shown above.



3.5.8.1. The Autofocus Procedure

The Autofocus procedure uses a software controlled motor to control the microscope focus. The focus position is adjusted in small increments whilst monitoring a signal from the sample. The sample signal is maximum at the focus position, and thus the focus adjustment is made iteratively until the maximum signal is observed. At this point, the Raman measurement is made.

There are two main motors which are used to control the microscope focus.

- Z motor – this motor controls the fine focus wheel of the microscope. Its maximum range is limited only by the microscope focus mechanism, and the available space between the objective and the sample.
- Piezo device (Pifoc) – this motor is attached to a single microscope objective, and uses piezo controllers to adjust the objective position up or down. Its maximum range is limited by the piezo controllers, and typically has a travel of approximately 100 µm. By using piezo motors, this device is extremely fast, and has very fine step size (typically <50 nm).

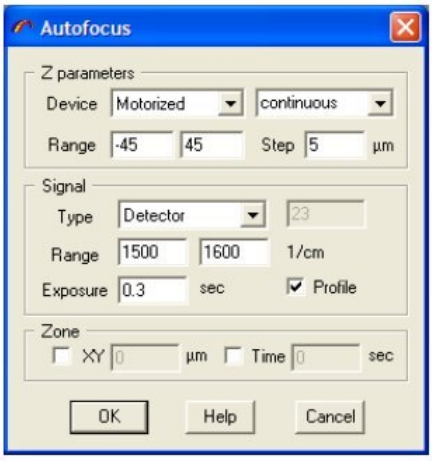
There are two main signals which can be detected to find the focus position.

- Reflection – this signal is the reflected and Rayleigh scattered light from the sample, and is detected by a small pin diode detector positioned at the top of the microscope, which monitors the total signal intensity (without spectral information). The signal will be greatest at the surface of the sample.
- Detector – this signal is based on the Raman signal from the sample detected in the normal way using the active detector. The signal will be greatest at (or just below) the surface of the sample.

The hardware present on your instrument will dictate whether autofocus is possible, and if so, what motor and signal will be used.

3.5.8.2. Setting Up Autofocus

The Autofocus dialog window (accessed *via* Acquisition > Autofocus) allows the Autofocus to be configured.



Z parameters

Select the motor type from the "Device" drop down box. Depending on the instrumentation configuration there may only be one motor available. The typical devices which are used are as follows:

- Motorized – this uses the Z motor connected to the fine focus wheel of the microscope.
- Pifoc – this uses the piezo Z axis motor attached to a specific objective.

Select the scan type from the adjacent drop down box. There are two types of autofocus scans which can be used:

- Continuous – the motor will make a continuous sweep through the Z axis, whilst reading out the signal.
- Step by step – the motor will move to a set position, then read out the signal; then move to the next position etc.

Set the Z scan "Range" and "Step". The range must be set sufficiently large to ensure that the sample's roughness can be countered using Autofocus; however the range must not be set so large that the objective would be driven into the sample. With the "Motorized" Z motor device the range is only limited by the microscope focus mechanism, and the available space between the objective and the sample. With the "Pifoc" piezo motor the range is limited by the piezo controllers, and typically has a travel of approximately 100 µm.

CAUTION: TAKE CARE SETTING THE Z SCAN "RANGE" WHEN USING THE Z MOTOR ("MOTORIZED" DEVICE). IF THE RANGE IS SET LARGER THAN THE AVAILABLE SPACE BETWEEN THE SAMPLE AND THE OBJECTIVE, THE SAMPLE, OBJECTIVE, MICROSCOPE AND/OR Z MOTOR COULD BE SERIOUSLY DAMAGED.

Signal

Select the autofocus signal from the "Signal" drop down box.

- Reflection - this signal is the reflected and Rayleigh scattered light from the sample, and is detected by a small pin diode detector positioned at the top of the microscope, which monitors the total signal intensity (without spectral information).
- Detector - this signal is based on the Raman signal from sample detected in the normal way using the active detector.

Tick the "Profile" box to create a profile of signal intensity *versus* Z (depth) position during the autofocus procedure. This can be a useful tool to monitor and troubleshoot the autofocus procedure.

If "Reflection" signal is selected, there are no other parameters which need to be set in the "Signal" section.

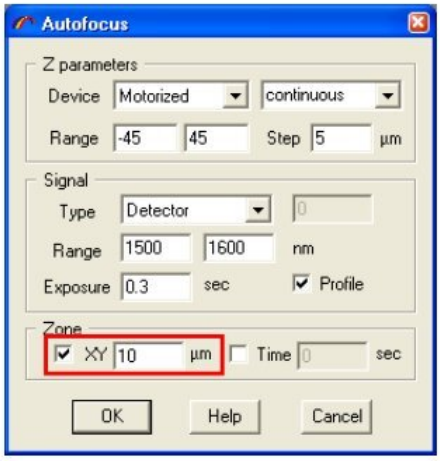
If "Detector" signal is selected, specify the spectral range which will be used to optimise the focus position, and set the "exposure" (acquisition) time (in seconds, s) which will be used for the autofocus spectrum read out. Note that this exposure time is used only for the autofocus procedure – once the focus position is located the sample measurement will be made using the acquisition parameters set in the Control Panel (see section 9.9, page 234).

Zone

The "Zone" section of the Autofocus dialog window can be used to set up an intermittent autofocus procedure. The "XY" mode sets the autofocus to be made after a fixed distance across the sample, whilst the "Time" mode sets the autofocus to be made after a fixed time.

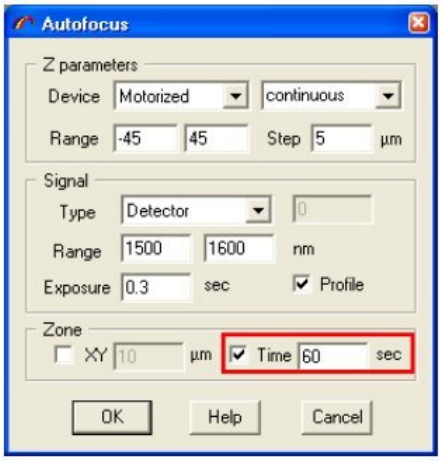
- XY – Tick the box for "XY", and type in the desired distance (in micrometers, µm) between autofocus operations.

  This mode is useful when the sample has a very gradual change in focus across its surface, and it is not necessary to autofocus at every map position.



- Time – Tick the box for "Time", and type in the desired time (in seconds, s) between autofocus operations.

  This mode is useful when the sample may slow move out of focus due to temperature fluctuations, and it is not necessary to autofocus at every point.



3.5.8.3. Turning On Autofocus

In Acquisition > Options, select the appropriate mode from the "Autofocus" drop down box (see section 3.5.4.8.1, page 40).

Click [**OK**]. Autofocus is now active.

The Autofocus indicator icon is displayed in the Status Bar. 

3.5.8.4. Setting the Autofocus Offset

In Acquisition > Options, type in the desired offset for the Autofocus position. A negative offset means that the focus will be moved upwards (e.g., analysis will be made higher in the sample than the

autofocus position); a positive offset means that the focus will be moved downwards (e.g., analysis will be made lower in the sample than the autofocus position). See section 3.5.4.8.2, page 40, for further information.

3.5.8.5. Turning Off Autofocus

In Acquisition > Options select "Off" from the "Autofocus" drop down box (see section 3.5.4.8.3, page 40).

Click **[OK]**. Autofocus is now inactive.

The Autofocus indicator icon is no longer displayed in the Status Bar.

3.5.9. Extra Images

The "Extra Images" dialog window allows extra images to be recorded using other detector devices on the system (such as the Autofocus "reflection" pin diode detector – see section 3.5.8, page 59). There are two main modes:

- Image – this allows for stand alone images to be recorded using the signal from the additional detector. The dimensions of the image (in X, Y and/or Z axes) and the number of image pixels are defined in the "Extra Images" dialog window.
- Map – this allows signal from the additional detector to be recorded simultaneously with a multidimensional spectral array measurement (such as a Raman XY surface map). The dimensions of the image (in X, Y and/or Z axes) and the number of data points are taken from the "Mapping Properties" dialog window (see section 4.5.5, page 97) and do not need to be set in the "Extra Images" dialog window.

3.5.9.1. What are Extra Images For?

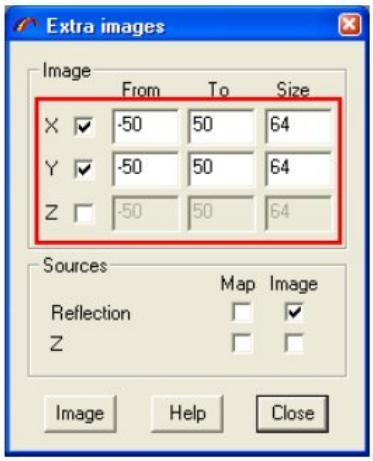
The "Extra Images" function can be useful to obtain additional information about a sample which wouldn't normally be possible on a Raman microscope.

One typical example is to create a map of the sample's surface topography (roughness). This can be done by using information obtained during the Autofocus procedure (see section 3.5.4.8, page 39). If the Autofocus procedure is used for a Raman XY map of the surface at each measurement point in the map the software has calculated a focus position based on an autofocus signal. These two values (focus position and signal) directly reflect the topography of the sample. The "Extra Images" function allows the topography image to be created simultaneously with the Raman map.

3.5.9.2. Setting Up an Image Acquisition

Tick the axis boxes to activate the X, Y and Z axes as required.

Set the image dimensions (in micrometers, µm) using the "From" and "To" boxes, and number of image pixels using the "Size" boxes.

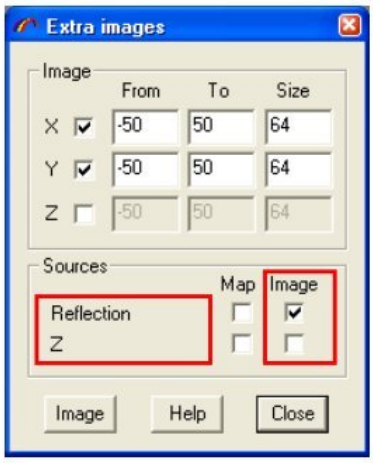


Select the signal source (additional detector) which will be used to create the image, by ticking the "Image" box(es) in the "Sources" section..

The most common sources are as follows:

- Reflection – autofocus pin diode detector.
- Z – Z-axis position of the piezo/Z motor.

Other sources may be visible in this dialog window, depending on the configuration of your system.

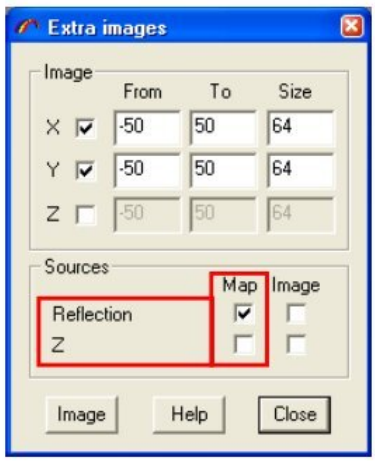


Click **[Image]** to start the acquisition.

3.5.9.3. Setting Up a Map Acquisition

Select the signal source (additional detector) which will be used to create the mapped image by ticking the "Map" box(es) in the "Sources" section.

Nothing needs to be set or adjusted in the "Image" section of the dialog window. Note that if all three image axes (X, Y and Z) are unticked, then the **[Image]** button will be greyed out. This button is not used for map acquisition.



Click **[Close]** to close the "Extra Images" dialog window.

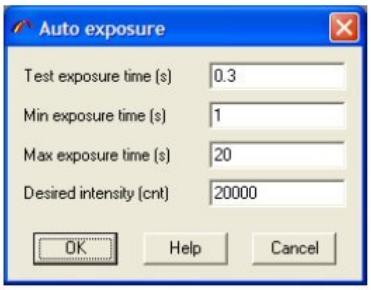
Set up the Raman multidimensional spectral array measurement in the normal way using the "Map Properties" dialog window (see section 4.5.5, page 97)

Start the Raman multidimensional spectral array measurement in the normal way using the "Mapping Acquisition" icon (/). An additional map image (or images) will be displayed as the Raman map proceeds.

3.5.10. Auto Exposure

The Auto Exposure function calculates the necessary acquisition time to acquire a spectrum with a maximum signal level specified by the user. It is useful to ensure a good quality spectrum is acquired regardless of the inherent signal strength of the sample.

The Auto Exposure function initially acquires a test spectrum with a fast 'test' acquisition time, and calculates the maximum signal level in the spectrum. Based on this data a new 'final' acquisition time is calculated to yield a maximum signal equal to the specified 'desired intensity', and the acquisition is made. 'Minimum' and 'Maximum' acquisition times are also set by the user, to prevent the software attempting an infinitely short or long measurement.



Test exposure time (s)
Set the initial test acquisition time, in seconds (s). Typically an acquisition time between 0.3s and 1s is suitable, although it can be increased if most samples are very weak Raman scatterers.

Minimum exposure time (s)
Set the minimum acquisition time which can be selected by the software for the final measurement. Typically an acquisition time between 0.1s and 0.5s is suitable.

Maximum exposure time (s)
Set the maximum acquisition which can be selected by the software for the final measurement.

For weak Raman scatterers the final acquisition time required to reach the 'Desired intensity' may exceed the 'Maximum exposure time'. In this case the final acquisition time will be equal to the 'Maximum exposure time' and the achieved intensity may be less than the 'Desired intensity'.

Desired intensity (cnt)
Set the desired maximum signal level (in counts, cnt) for the final spectrum, ensuring that it is below the saturation point of the detector being used. Remember that the larger the desired intensity the longer the measurement will take, but the better the spectrum quality.

For weak Raman scatterers the 'Desired intensity' may not be achieved because the final acquisition time required to reach this intensity would need to exceed the 'Maximum exposure time'. In this the 'Maximum exposure time' is used.

3.5.10.1. Turning the Auto Exposure Function On and Off

The Auto Exposure function is turned on and off using the "Autoexposure" drop down box in Acquisition > Options – see section 3.5.4.12, page 42.

When Auto Exposure is active, the Acquisition Time section in the Control Panel is set to "Auto" and greyed out.

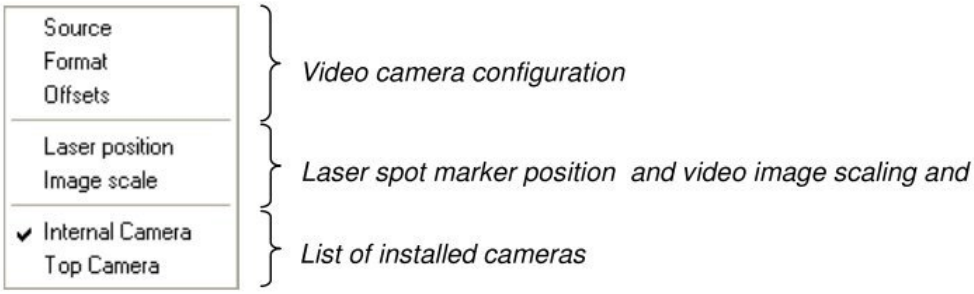
3.5.11. Heat Detector

For peltier cooled (liquid nitrogen free) detectors, the "Heat Detector" function will switch off the peltier cooling and allow the detector to warm up to room temperature.

Cooling can be restarted either by setting the temperature in Acquisition > Detector (see section 3.5.7.2, page 59), or by exiting and restarting LabSpec 5.

3.6. Video

The Video menu allows configuration of the instrument's video camera(s), set up of video image scaling and the laser spot marker position, and (when multiple cameras are present) selection of the active camera.



CAUTION: CHANGING PARAMETERS ACCESSED THROUGH THE VIDEO MENU COULD CAUSE THE VIDEO CAMERA(S) TO CEASE WORKING, OR TO WORK INCORRECTLY. YOU SHOULD ONLY MODIFY PARAMETERS IN THE VIDEO MENU WHEN INSTRUCTED TO DO SO BY A TRAINED ENGINEER FROM HORIBA SCIENTIFIC OR ONE OF ITS REPRESENTATIVES.

3.6.1. Video Camera Configuration

The dialog windows for "Source", "Format" and "Offsets" allow the configuration of the video camera(s) to be set up, or modified. The dialog windows will vary depending to the specific camera(s) installed on the instrument.

Please consult your local HORIBA Scientific engineer or representative to find out more details about the configuration options available for the cameras on your system.

3.6.2. Laser Spot Marker Position and Video Image Scaling

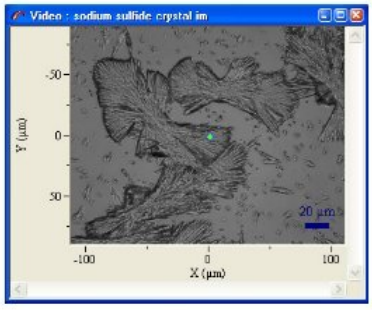
The "Laser Position" and "Image Scale" options allow the position of the laser spot marker on the video image, and the scaling of the video images to be set.

3.6.2.1. Laser Position

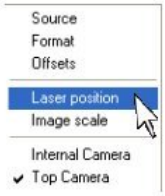
Click on Video > Laser position to set the position of the green laser spot marker on the video image.

3.6.2.1.1. Setting the Laser Spot Marker Position

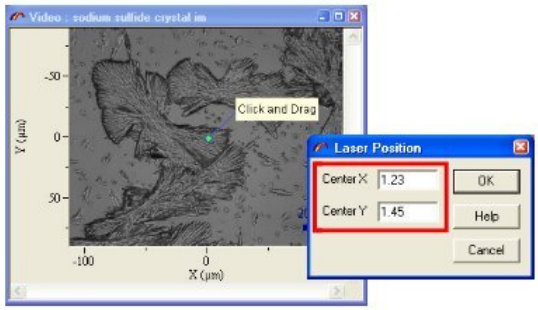
Acquire a video image, using the video icon (/). Ensure that the objective selected in the Control Panel (see section 9.7, page 233) corresponds to the objective being used on the microscope.



Click on Video > Laser position.



Drag the green laser spot marker to the desired position on the video image, or type the desired coordinates into the "Center X" and "Center Y" boxes.



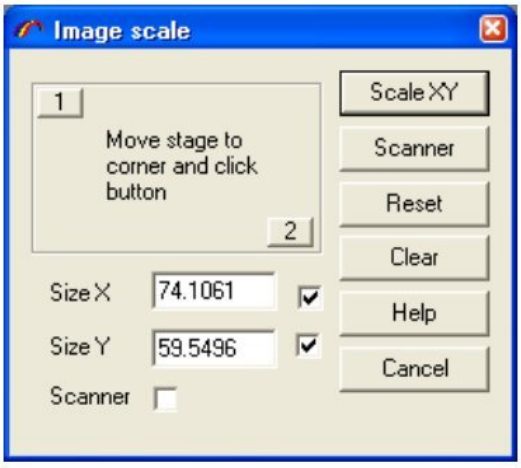
Click **[OK]**. The laser spot marker is now repositioned.

Note that this process is objective specific – the laser spot marker position should be set for each objective on the microscope.

3.6.2.2. Video Image Scale

Click on Video > Image scale to open the "Image scale" dialog window.

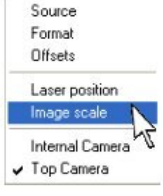
A resizeable scale box will additionally appear on the video image. The scale box can be resized to a known size, and the known values used to scale the video image.



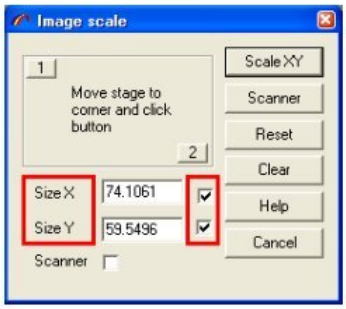
3.6.2.2.1. Setting the Video Image Scale Using the "1" "2" Corner Positions

Acquire a video image, using the video icon (/). Ensure that the objective selected in the Control Panel (see section 9.7, page 233) corresponds to the objective being used on the microscope.

Click on Video > Image scale to open the "Image scale" dialog window.

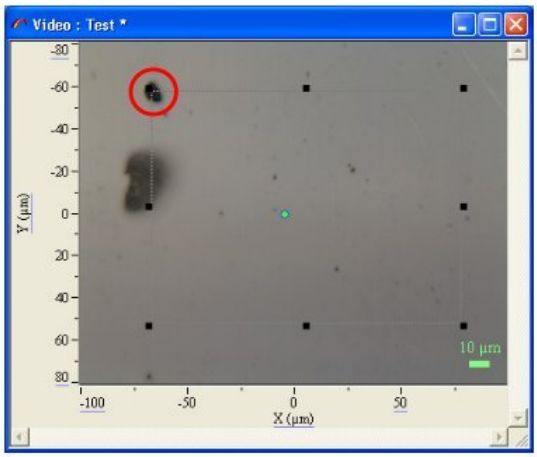


Ensure the “Size X” and “Size Y” boxes are ticked.

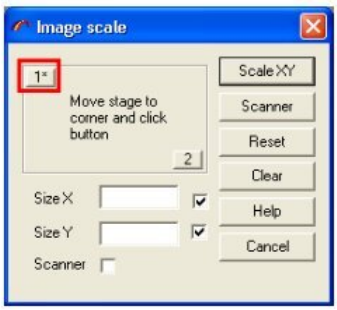


Drag the scale box to cover a large proportion of the video image.

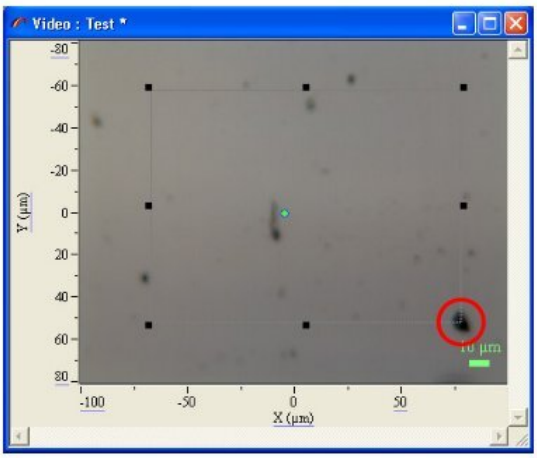
Move a small, easily recognisable feature of the sample so that it is positioned exactly at the top, left hand corner of the scale box. Dust particles on the Silicon calibration sample are ideal for this, but any sample with recognisable features can be used.



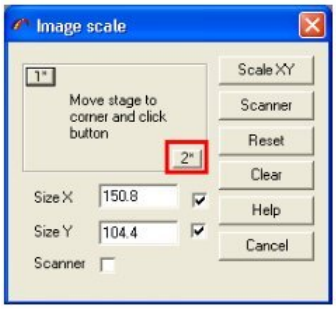
Click **[1]**. The scale values in the “Size X” and “Size Y” boxes are cleared.



Using the joystick move the XY stage so that the feature is positioned exactly at the bottom, right hand corner of the scale box.



Click **[2]**. The scale values in the "Size X" and "Size Y" boxes are set according to the distance moved by the XY stage.



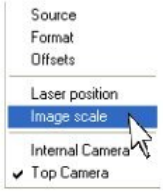
Click **[Scale XY]** to register the video scale.

Note that this process is objective specific – the video image scale should be set for each objective on the microscope.

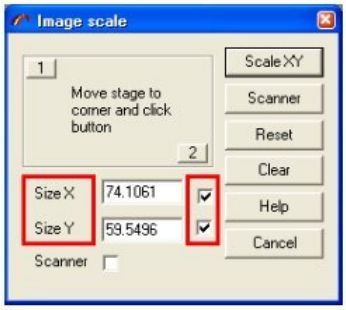
3.6.2.2.2. Setting the Video Image Scale Manually

Acquire a video image, using the video icon (/). Ensure that the objective selected in the Control Panel (see section 9.7, page 233) corresponds to the objective being used on the microscope.

Click on Video > Image scale to open the "Image scale" dialog window.

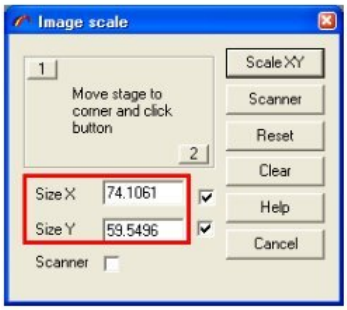


Ensure the "Size X" and "Size Y" boxes are ticked as appropriate. With this procedure it is possible to scale just one dimension (X or Y) if desired. The dimension(s) to be scaled must be ticked.



Drag the scale box (in X and/or Y dimensions, as appropriate) to match a feature of known size. Typically this will be a graticule scale bar, but any sample feature of precisely known size can be used.

In the "Size X" and/or "Size Y" boxes type in the size of the feature to which the scale box has been match



Click **[Scale XY]** to register the video scale.

If only one dimension (X or Y) has been scaled in this manner, ensure that the other dimension is scaled using a similar process. Both dimensions (X and Y) must be scaled to have correctly scaled video images.

Note that this process is objective specific – the video image scale should be set for each objective on the microscope.

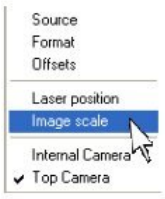
3.6.2.2.3. Setting the Scanner Scale

The Scanner Scale function is used to set the scan range of the confocal LineScan mirror.

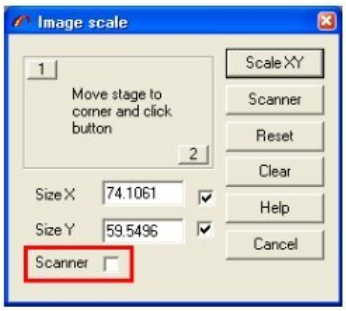
Acquire a video image, using the video icon (/). Ensure that the objective selected in the Control Panel (see section 9.7, page 233) corresponds to the objective being used on the microscope.

Turn on the laser and/or open the shutter, so that the laser beam is visible in the video image.

Click on Video > Image scale to open the "Image scale" dialog window.



If the system has a software controlled motorized Scanner, tick the "Scanner" box to start the Scanner motorized mirrors; otherwise ensure that the scanner is started using the switch on the instrument control unit. The laser beam will now be rastored on the sample in the Y direction.



Drag the scale box (in the Y dimension) to match the scan height of the rastored laser beam.

Click **[Scanner]** to register the video scale.

Note that this process is objective specific – the video image scale should be set for each objective on the microscope.

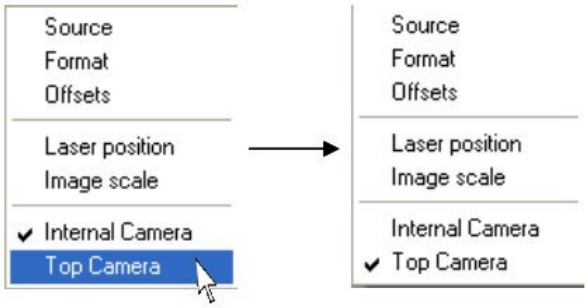
3.6.3. List of Installed Cameras

A list of all installed cameras is displayed at the bottom of the Video menu. The currently active camera is denoted by a tick next to its name – this camera will be used when the video read out is started with the camera icon (/).

3.6.3.1. Selecting a Camera

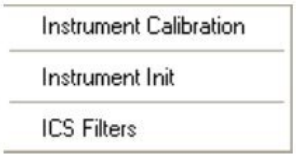
The currently active camera is denoted by a tick next to its name – this camera will be used when the video read out is started with the camera icon (/).

To change the active camera, click on the camera name listed in the Video menu.



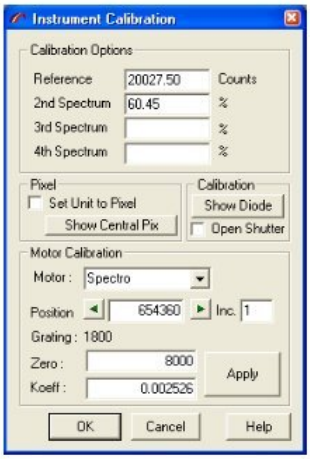
3.7. Setup

The Setup menu provides access to some specific functions for hardware control.



3.7.1. Instrument Calibration

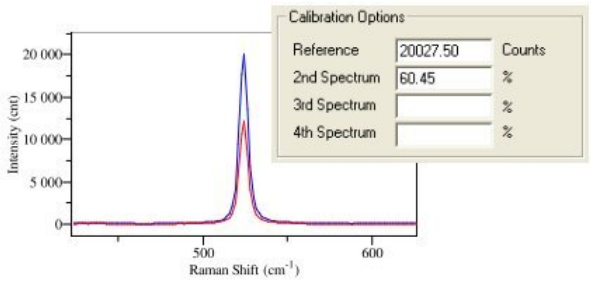
The "Instrument Calibration" dialog window allows the user to perform a manual calibration of the spectrometer, and provides additional useful information and control for general maintenance of the system.



3.7.1.1. Calibration Options

The "Calibration Options" section displays the maximum signal level of the active spectrum, and the relative intensity of the next three spectra displayed in the spectrum window.

In the example shown right, the active spectrum (—) has a maximum intensity of 20027.50 counts, whilst the second spectrum (—) has a maximum intensity of 12105.90 counts, which is 60.45% of the active spectrum intensity.



3.7.1.2. Pixel

Tick the "Set Unit to Pixel" to display spectra with X axis units of detector pixels, rather than the units selected in Options > Unit. This can be useful to locate the position of a hot pixel on the detector.

Click on **[Show Central Pixel]** to mark the central pixel position on the spectrum.

3.7.1.3. Calibration

Click on **[Show Diode]** to move the spectrometer to the pre-set internal diode position. This position allows the internal reference diode to pass through the spectrometer and confocal optics onto the sample. The internal reference diode can be used for back alignment of the spectrometer and optics.

Tick the "Open Shutter" box to force the detector shutter open, so that the internal reference diode spot can be viewed on the sample.

3.7.1.4. Motor Calibration

The "Motor Calibration" section allows the reference positions of the instrument's motors to be set. The most common use of this section is to perform a manual calibration of the spectrometer ("spectro" motor).

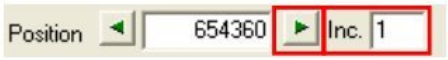
CAUTION: CHANGING PARAMETERS IN THE "MOTOR CALIBRATION" SECTION COULD CAUSE THE INSTRUMENT TO CEASE WORKING, OR TO WORK INCORRECTLY. YOU SHOULD ONLY MODIFY PARAMETERS IN THE "MOTOR CALIBRATION" SECTION AFTER TRAINING BY A TRAINED ENGINEER FROM HORIBA SCIENTIFIC OR ONE OF ITS REPRESENTATIVES.

For a step by step guide to manual calibration of the spectrometer please see section 10, page 247.

Motor
Select the motor to be calibrated from the "Motor" drop down box.

Position
The current position of the selected motor is displayed in motor step units.

Click on the left hand ar row to sequentially move the motor position to lower position. The position will be reduced by the increment shown in the "Inc." box.



Click on the right hand arrow to sequentially move the motor position to higher position. The position will be increased by the increment shown in the "Inc." box.

Grating
The selected diffraction grating (see section 9.6.1, page 231) is displayed.

Zero:
The "Zero" number is the motor step position for the end (zero) position of the motor.

For the spectrometer, "Zero" corresponds to the motor step position at which the diffraction grating is at zero order (=0 nm).

Koeff:
The "Koeff" number relates the function of the motor to its step size.

For the spectrometer, "Koeff" corresponds to the number of nanometers (nm) travelled per motor step.

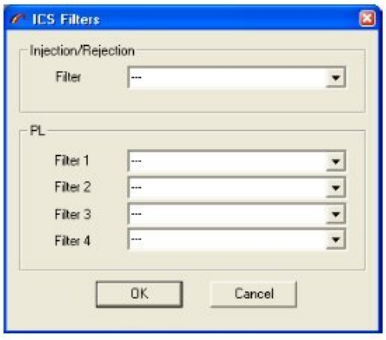
Apply
Click **[Apply]** to register a change in the "Zero" or "Koeff" values.

3.7.2. Instrument Init

Clicking on Setup > Instrument Init will re-initialize the instrument. This means that all motor positions will be returned to their reference position, and then sent to the positions indicated in the software.

3.7.3. ICS Filters

Clicking on Setup > ICS Filters opens the ICS Filters dialog window. This allows a specific filter set to be specified so that an appropriate "HORIBA ICS" intensity correction factor will be applied for measurements made with this filter set. Full information about automatic intensity correction and the "HORIBA ICS" correction factor can be found in section 3.5.4.14, page 43.



3.7.3.1. Selecting a Specific Filter for Intensity Correction

Click on Setup > ICS Filters to open the ICS Filters dialog window.

Select the required laser injection/rejection filter from the "Injection/Rejection" section, or a combination of photoluminescence (PL) filters from the "PL" section.

Note that an intensity correction factor must have been created for the specified filter combination, otherwise an error message similar to that shown right will be displayed when an acquisition is started. Click on **[OK]** or **[Cancel]** to clear the message, and start the acquisition. Remember that in this case the spectrum will be uncorrected. The error message will only be shown once during a LabSpec session – if LabSpec is closed and then re-opened, the message will be displayed again if intensity correction is activated when no intensity correction factor has been created.

3.8. LabAssistant

The LabAssistant menu is the interface for the LabSpec 5 user assistance module, and allows definition and use of hardware/software templates and the GO! (Guided Operation) Wizard.

3.8.1. LabAssistant Templates

The LabAssistant templates provide enhanced template control compared with the Options > Template function (see section 3.4.6, page 29). It is recommended that the LabAssistant function is used in preference to Options > Template.

Templates allow you to save a specific instrument configuration and measurement set up. The template can be recalled and applied as required. Templates are useful when you wish to make a number of sample types, each of which requires a specific measurement configuration. In this case, a number of templates can be set up allowing you to quickly recall the desired configuration without needing to set each parameter individually.

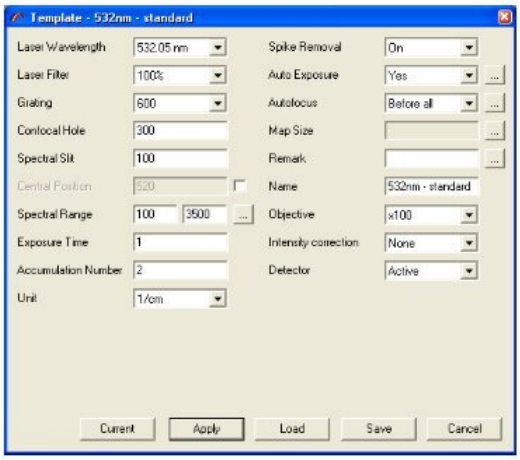
To learn more about the various options shown in the template dialog window please consult the relevant section of this manual.

3.8.1.1. Default Templates

Hover the mouse over LabAssistant > Default templates to see a list of default templates installed with the system.

Click on the desired template name to open the template dialog window.

Note that all modules and accessories active on the system will be visible within the "Template" window. Depending on your instrument configuration the "Template" window you see may vary from that shown here.



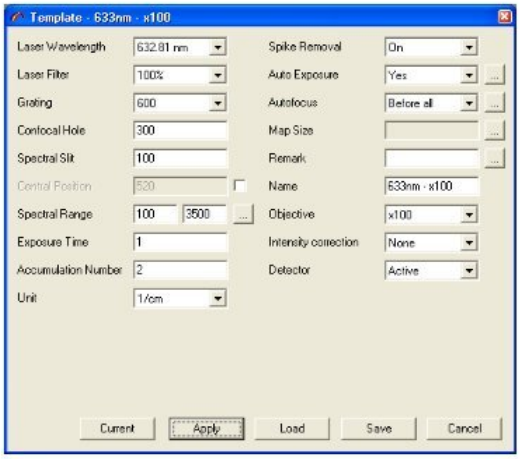
Click **[Apply]** to apply the current template – motorized hardware changes will be made automatically; manual hardware changes will prompt for you to change the hardware.

3.8.1.2. Custom Templates

Hover the mouse over LabAssistant > Custom templates to see a list of user defined custom templates installed with the system. Please see below for details about creating (section 3.8.1.3, page 78) and managing (section 3.8.1.4, page 79) custom templates.

Click on the desired template name to open the template dialog window.

Note that all modules and accessories active on the system will be visible within the "Template" window. Depending on your instrument configuration the "Template" window you see may vary from that shown here.



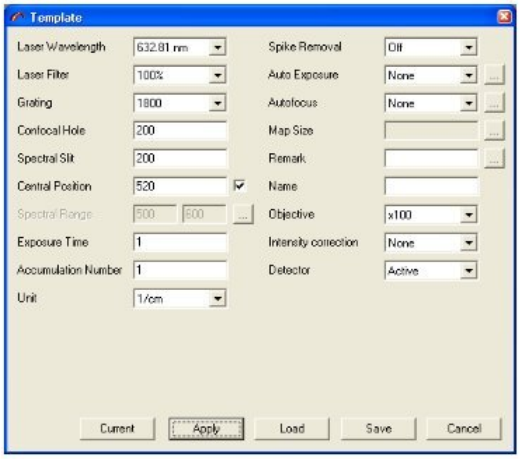
Click **[Apply]** to apply the current template – motorized hardware changes will be made

automatically; manual hardware changes will prompt for you to change the hardware.

3.8.1.3. Create Template

To create a new user defined custom template click on LabAssistant > Create template.

The template dialog window will be opened, showing a default configuration.

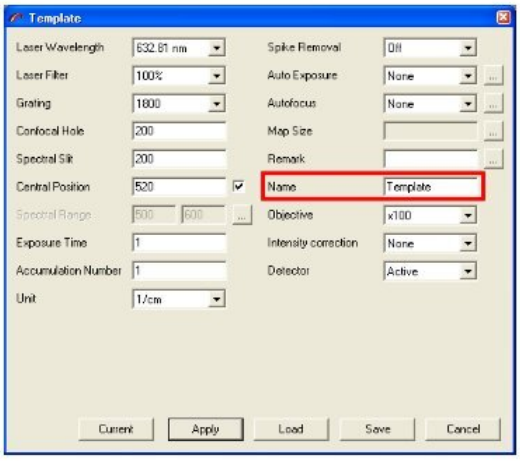


Click on **[Current]** to import the current instrument configuration and measurement set up into the Template.

Click **[Apply]** to apply the current template – motorized hardware changes will be made automatically; manual hardware changes will prompt for you to change the hardware.

3.8.1.3.1. Saving a Template

To save the template, type a name into the "Name" box, and click **[Save]**.



The template will be saved (in .tpl format) in a designated folder according to the user's profile (see section 3.4.5, page 25). The folder name will be the user's log-in name.

If "Profiles..." is not active, or if a guest user is logged in the template will be saved into a folder named Guest.

3.8.1.3.2. Opening an Existing Template

To open an existing template click **[Load]**. A file browse window will be opened.

Locate the template to be opened, and click **[Open]**.

Note that even if a template has been opened from another user's folder, the save location will default to the current user's folder. This ensures that templates created by another user cannot be overwritten or modified, except by the original creator.

3.8.1.4. Manage Templates

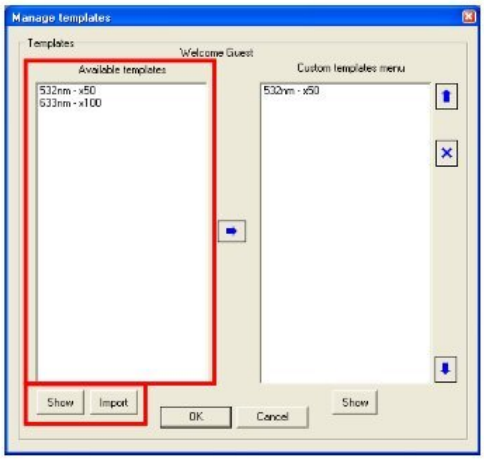
Click on LabAssistant > Manage templates to open the "Manage Templates" dialog window, and organize the templates. The log-in name of the current user is shown at the top of the window. If user "Profiles..." are not active, or if a guest user is logged in, the log-in name will be "Guest".

3.8.1.4.1. Available Templates

A list of all templates available to the current user is shown in this box.

Click on a template name, and then click **[Show]** to open the template dialog window to view the template configuration.

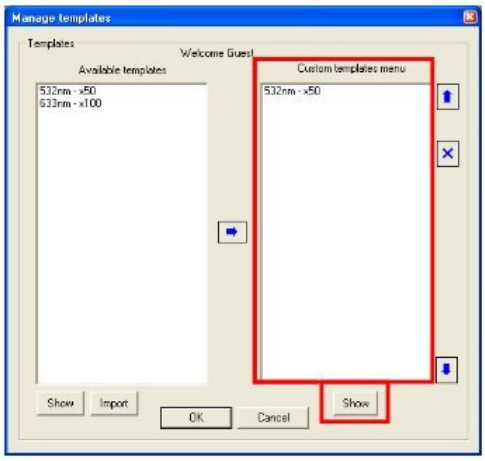
Click on **[Import]** to import a template from another user's folder into the current user's template location.



3.8.1.4.2. Custom Templates Menu

A list of templates which will be displayed in LabAssistant > Custom templates is shown in this box.

Click on a template name, and then click **[Show]** to open the template dialog window to view the template configuration.

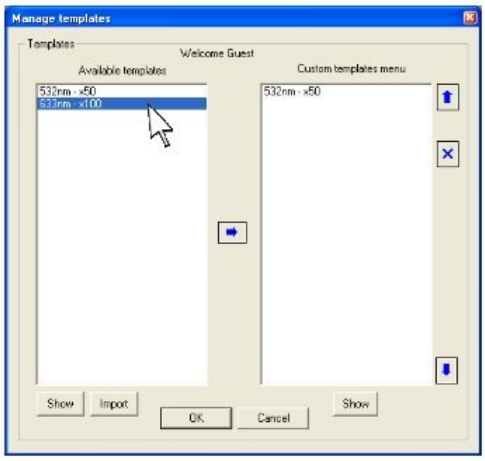


3.8.1.4.3. Organizing the Templates

Templates can be organized according to the user's preference.

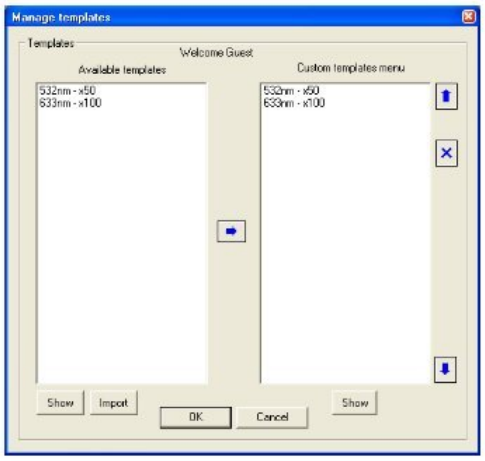
Show a Template in the Custom Templates Menu

To make an available template show in LabAssistant > Custom templates, click on the template in the "Available templates" box.



Click on the "transfer" button.

The selected template is now copied to the Custom Templates Menu box.



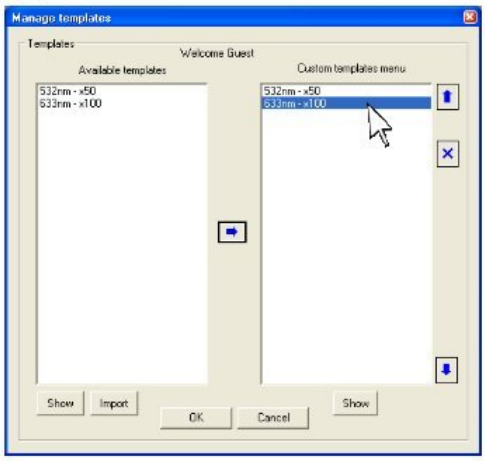
Modify the Display Position of a Template

To change the display position of a template name in the LabAssistant > Custom templates menu, click on the template name (in the Custom templates menu box) which is to be moved.

Click on the "Move Up" or "Move Down" buttons to move it up or down in the list displayed in the Custom templates menu box.

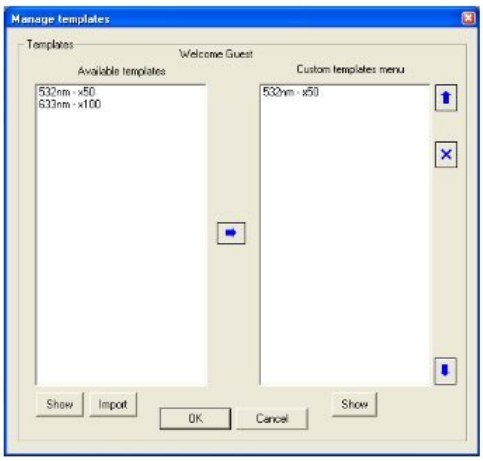
Remove a Template from the Custom Templates Menu

To remove a template from the LabAssistant > Custom templates menu, click on the template name (in the Custom templates menu box) which is to be removed.



Click on the "Remove" button.

The template name is removed from the Custom Templates Menu box. Note that this action does not actually delete the template. It is still listed in the Available Templates box.



3.8.2. G.O. (Guided Operation Wizard)

The Guided Operation (GO!) wizard is intended to assist less experienced users, who may not be familiar with the technical configuration of a Raman system, and may not understand how key hardware and software parameters affect the measurement result.

The GO! wizard leads the user through the experiment set up with a series of questions, and provides helpful, real life hints as to which option should be chosen. At the end the chosen parameters are applied, and the measurement is then made.

3.8.2.1. Using the Guided Operation Wizard

Click on LabAssistant > G.O. to open the GO! Wizard start up window.

Click **[Start GO]** to start the wizard with the first question.

Hover the mouse over the HINTS panel to view hints about the choice of parameter. In some cases additional information can be accessed using the hyperlinks within the hint text.

Move the mouse away from the HINTS panel to hide the hint text, and select the parameter listed in the GO! dialog window.

3.9. Scripts

The Scripts menu offers Visual Basic Scripting (VBS) functionality for LabSpec 5, which allows specific hardware control, data acquisition and data analysis functions to be written by the user.

Open the VBS options and management window

Script menu

The VBS functionality and command set is not included in this manual, and users are referred to the document "LabSpec_5_Activex-VBS_documentation.pdf" located in the folder C:\Program Files\NGSLabSpec\.

3.9.1. Script Options and Management Window

Click on Scripts > Options to open the script options and management dialog window.

3.9.1.1. Scripts

The "Scripts" section allows VBS scripts (in .vbs format) to be loaded and edited.

Multiple scripts can be loaded, and are displayed in the "Script:" drop down box. Use the drop down box to select a loaded script.

The file path of the selected script is displayed in the "Path:" section.

Load a Script

Click on the "Add Script to List" button to load a VBS script into the "Script:" drop down box.

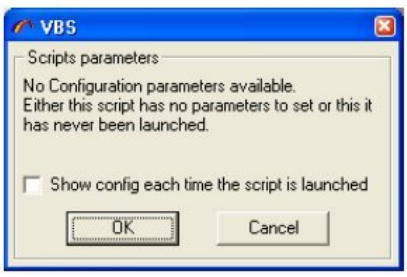
Remove a Script

Click on the "Remove Script from List" button to remove a VBS script from the "Script:" drop down box.

Configure a Script

Certain VBS scripts have a configuration interface where specific properties can be set. Click on the "Configure Script" button to open the selected script's configuration window.

If there are no configuration parameters for the selected script, a warning message will be displayed. Click **[OK]** or **[Cancel]** to clear the message.



Start Script

Click on the "Start Script" button to run the selected VBS script.

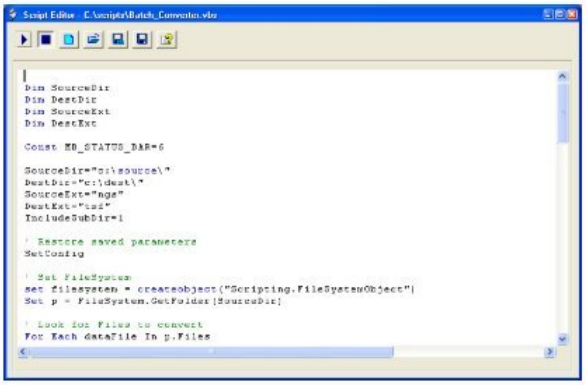
Open Script in Editor

Click on the "Open Script in Editor" button to open the selected VBS script in an editor window. See section 3.9.1.1.1, page 84).

3.9.1.1.1. Editing Scripts

VBS scripts can be edited directly in LabSpec 5 using the script editor tool. To launch the script editor, click on the "Open Script in Editor" button in the script options and management dialog window.

If a script is selected it will be opened in the editor window. If there are no scripts loaded into the "Script Options and Management" dialog window the editor window will be blank, allowing direct coding of a new script.



Start a Script

Click on the "Start Script" button to run the VBS script displayed in the editor window.

Stop a Script

Click on the "Stop Script" button to stop a VBS script which is running.

Create a New Script

Click on the "New Script" button to open a blank VBS script in the editor window.

Save a Script As

Click on the "Save Script As" button to save the current VBS script with a new name. The standard Windows file save dialog window will be opened.

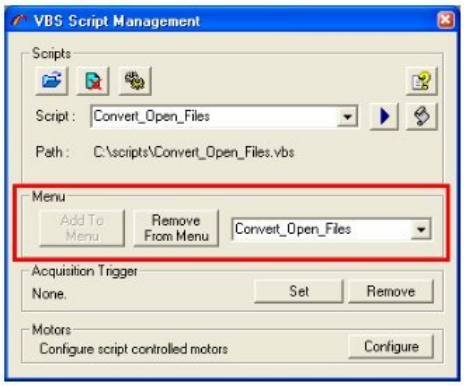
Save a Script

Click on the "Save Script" button to save the current VBS script with its existing name. No file save dialog window will be displayed.

3.9.1.2. Menu

The "Menu" section allows scripts loaded in the script options and management window to be displayed in the Scripts menu, from where they can be run through a single mouse click.

Scripts which are shown in the Scripts menu will be displayed in the "Menu" drop down box.



Add a Script to the Scripts Menu

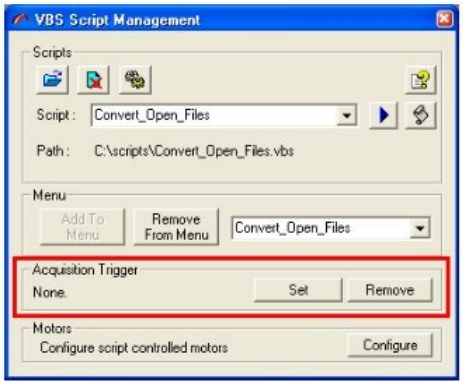
Select a loaded VBS script in the "Script:" drop down box, and click on the "Add To Menu" button to display the script name in the Scripts menu.

Remove a Script from the Scripts Menu

Select a script from the "Menu" drop down box, and click on the "Remove From Menu" button to remove the script name from the Scripts menu.

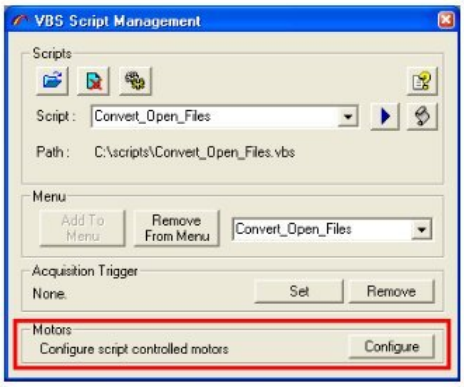
3.9.1.3. Acquisition Trigger

Click **[Set]** to set the current script (displayed in the Script drop down box) to be used as a trigger script. A trigger script is one which will be applied immediately before or after an acquisition.



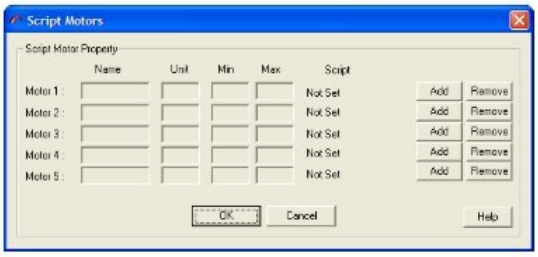
3.9.1.4. Motors

Click on **[Configure]** to open the Script Motors dialog window.



3.9.1.4.1. Script Motors Dialog Window

The Script Motors dialog window allows external motors not usually controlled by LabSpec 5 to be controlled using a VBS script.



Up to five motors can be configured. Click **[Add]** to open a standard file open dialog window to browse for a script.

Type in the motor name, unit of motor movement (such as 'steps' or 'micrometers'), and maximum and minimum motor values.

Click **[Remove]** to remove a script from the list.

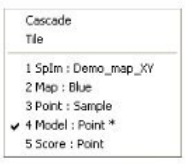
3.9.2. Script Menu

A list of VBS script names can be displayed in the Scripts menu. Clicking on a script name will automatically run the script.

To add and remove scripts from the Scripts menu please see section 3.9.1.2, page 85.

3.10. Window

The Window menu allows fast arrangement and selection of the data display windows in LabSpec 5. This menu item is only active and visible when data is open.



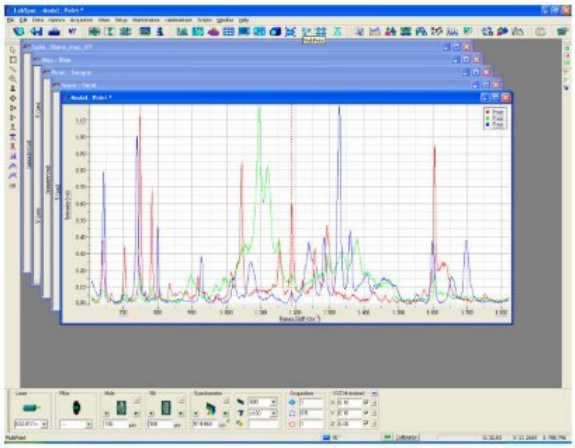
Window display mode

List of current display windows

3.10.1. Window display mode

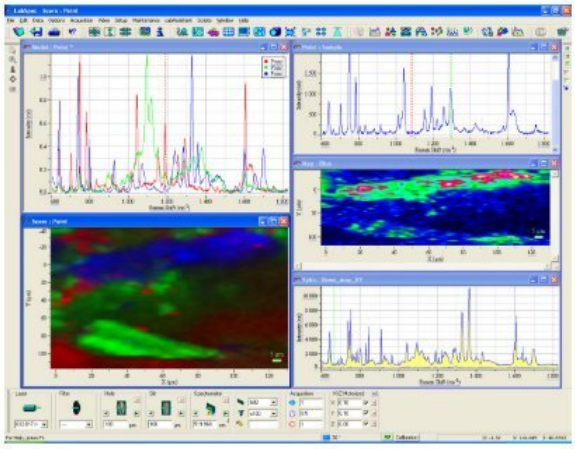
Cascade

Click on Cascade to display all open data windows in cascade mode. The windows can still be manually positioned and sized.



Tile

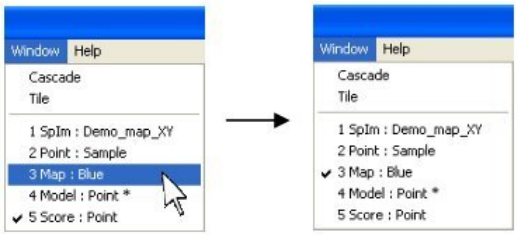
Click on Tile to display all open data windows in tile mode – in this mode, the windows will be positioned and sized so that all open windows are visible. The windows can still be manually positioned and sized.



3.10.2. List of current display windows

The list of current display windows shows all current data windows open in LabSpec 5. The active window title is ticked in the list.

To activate a window, left click the window title in the list. This window title will now be ticked.



3.11. Help

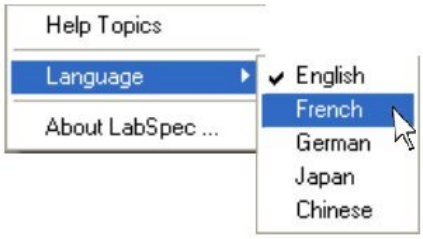
The Help menu provides access to the LabSpec 5 context help files and useful information about the installed version of LabSpec 5. In addition it is possible to select a language for the LabSpec 5 graphical user interface (GUI).

3.11.1. Help Topics

Click on Help > Help Topics to display the LabSpec 5 context help.

3.11.2. Language

Click on Help > Language to select a language for the LabSpec 5 graphical user interface (GUI).

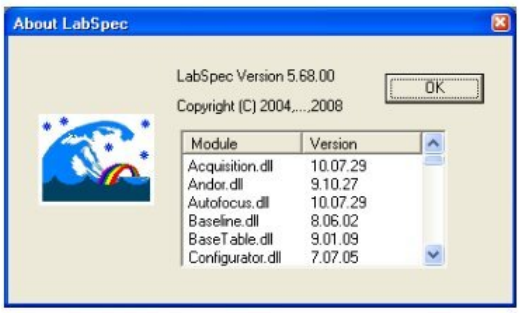


3.11.3. About LabSpec...

Help > About LabSpec... displays information about the installed version of LabSpec 5 and its individual modules (.dll files).

This information can be useful for troubleshooting, and it is advised that you make a note of the LabSpec version number (i.e., 5.XX.YY) prior to contacting HORIBA Scientific with regard to the software.

Click **[OK]** to close the information window.



4. Icon Bar

The Icon Bar located beneath the Menu Bar provides access to either standard Windows functions (such as File Open, File Save) or to specialized data acquisition/analysis modules and options. Some icons can also be activated using keyboard shortcuts – these shortcuts are indicated in the description of each icon, below. The Icon Bar(s) will *only* show icons which apply to the correctly installed and configured hardware/software options for your instrument.

The image below shows all of the standard icons, but be aware that some of these may not be visible in your software.

Big icons

Small icons

4.1. Icon View and Grouping

Please note that there are two icon views used in LabSpec 5, called “Big icon” and “Small icon”. In the descriptions that follow, the “Big icon” will be displayed first, followed by the “Small icon”.

4.1.1. Switching Icon View

To switch the icon view, right click on the icon tool bar. The current icon view will be ticked.

Click on the desired icon view (“Small icon” or “Big icon”).

The icon view will switch to the selected view.

4.1.2. Icon Grouping

The icons are grouped according to general functionality:

- Delete data
- Data management
- Cursors and data information

- Data acquisition
- Data processing and analysis
- Stop active function

Each group separated indicated by a thick vertical bar on its left hand edge (indicated right with ↑).

Sub-sections within a group are separated by a thin vertical bar (indicated right with ↑).

4.1.2.1. Moving Icon Groups

Icon groups can be moved by clicking and dragging on the thick vertical bar to the left hand side of the group. When the cursor changes to a double headed arrow the icon group can be dragged to a new position on the icon bar.

4.2. Delete Data Icon

4.2.1. Delete

Close the currently active data file from LabSpec. If the file has been modified since the last save, a standard Windows dialog window will prompt the user to save the file.

This icon closes data displayed within LabSpec, but does not delete saved data from the computer hard drive.

4.3. Data Management Icons

4.3.1. Open

Open a previously saved data file using a standard Windows dialog window. See section 3.1.1.1, page 10, for full details concerning available file formats.

This icon can be activated with the <CTRL>+O keyboard shortcut.

4.3.2. Save

Save the data file currently active in LabSpec 5. See section 3.1.1.1, page 10, for full details concerning available file formats and options for saving.

This icon can be activated with the <CTRL>+S keyboard shortcut.

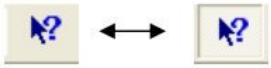
4.3.3. Print

Print the current data window using the Print Template. Full information about using and setting up the Print Template can be found in section 3.1.2.1, page 13.

This icon can be activated with the <CTRL>+P keyboard shortcut.

4.3.4. Help

Activates the context help function. When clicked this icon will be locked down. To release, click on the icon again.



The standard cursor will change to the "help" cursor – click on any icon to view its context help file.

4.4. Cursors and Data Information Icons

4.4.1. Scale Normalization

Rescale the active window so that all data is visible. In normal operation all axes are affected, but note that an axis with scaling set to "Freeze" or "Fixed" will not be rescaled. To rescale an axis ensure that "Freeze" or "Fixed" scaling is turned off (see section 8.1, page 204).

This icon can be activated using the right mouse click menu "Rescale" item (see section 8.3, page 209) or with the <CTRL>+N keyboard shortcut,

4.4.2. Intensity Normalization

Rescale the intensity axis of the active window. Note that if the intensity axis has scaling set to "Freeze" or "Fixed" will not be rescaled. To rescale an axis ensure that "Freeze" or "Fixed" scaling is turned off (see section 8.1, page 204).

4.4.3. Center Cursors

Center the active cursor(s) in the active window.

This function is useful when cursors are not visible in a window, because their position lies outside the range of the window. Click on this icon and the cursor(s) will be immediately visible in the center of the window.

This icon can also be activated by using the right mouse click menu "Center cursor" (see section 8.7, page 212).

4.4.4. Data Range

Open the "Data Range" dialog window, which displays information about the number of data points and maximum and minimum values for the active data. Typical information includes maximum and minimum values for the spectrum axis, intensity axis, XYZ spatial axes, and colors. The exact categories which are displayed depend on the specific 'type' of the selected data.

See section 3.3.2, page 20, for full information.

This icon can be activated with the <CTRL>+D keyboard shortcut, or through the Data > Data range... menu item.

4.4.5. Information

Open the parameters information dialog window.

This icon can be activated with the <CTRL>+I keyboard shortcut.

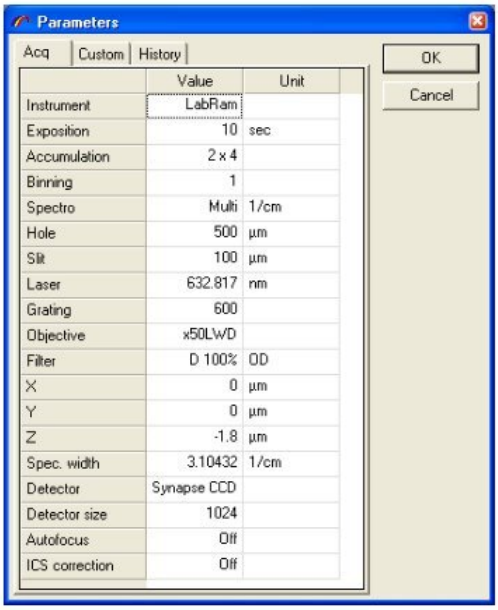
4.4.5.1. Parameters Information

The "Parameters Information" dialog window contains a wide range of information relating to the acquisition and processing of the active data. For example, it displays hardware set up (e.g., laser wavelength, confocal hole, diffraction grating and objective), acquisition parameters (e.g., acquisition time, number of accumulations, intensity correction), custom information provided by the operator (such as operator name, laser power, sample name), and a history of data processing steps made on the data.

Hardware, acquisition and processing history information is automatically filled in by LabSpec 5. Custom information needs to be filled in by the operator.

For information regarding the parameters displayed in the "Parameters Information" window, please see the relevant section of this manual.

Please note that the parameters displayed in the "Parameters Information" window will depend on the specific instrument configuration. The image below shows a typical view of the "Parameters Information" window, but be aware that some of these may not be visible in your software.

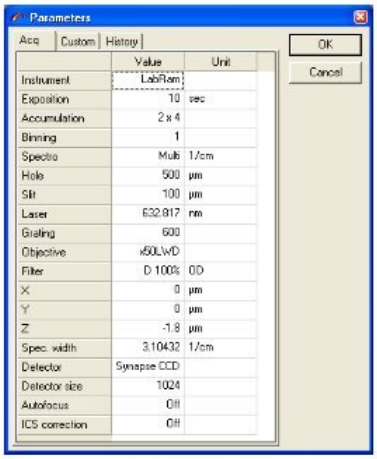


The information displayed in the "Parameters Information" window is useful to confirm how a specific measurement was made, and whether the data being viewed has been processed in some way.

Note that the parameters information is only retained when data is saved in a LabSpec file format – see section 3.1.1.1, page 10, for full information about available file formats. Data saved in LabSpec 4 (previous generation) format may not retain information about all parameters.

4.4.5.2. Acquisition Parameters Information

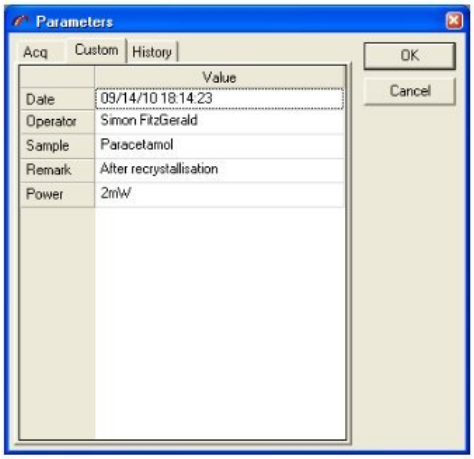
The "Acq" tab of the "Parameters Information" dialog window displays information relating to the hardware and software configuration used for the acquisition of the active data.



4.4.5.3. Custom Parameters Information

The "Custom Parameters" tab allows you to add specific information to a data file's "Parameters Information".

The Date field is automatically filled at the time of acquisition. The other fields are blank by default, but can be filled by the operator if desired.



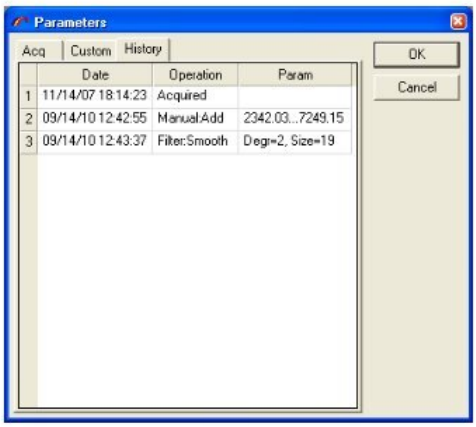
The Custom Parameters information tab can also be accessed through Acquisition > Custom info. In this case, the "Acq" and "History" tabs are not displayed.

For full information about using the Custom Parameters information window and how to customize it, please see section 3.5.1, page 30.

4.4.5.4. History Information

The "History Parameters" tab shows data processing operations made to the file since its acquisition.

The "Operation" field shows what processing function has been applied, and the "Param" field shows (where applicable) what parameters have been used for that function.



4.5. Data Acquisition Icons

4.5.1. Spectrum RTD (Real Time Display)

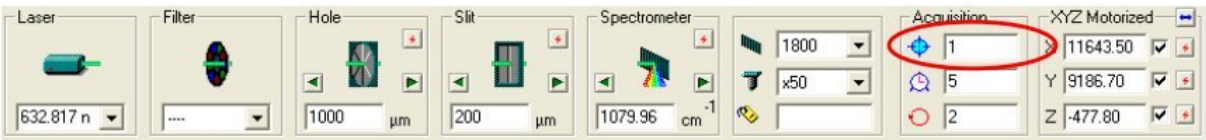
Start a spectrum RTD acquisition.

The spectrum real time display (RTD) provides a continuous spectrum readout of the detector. There is no accumulating or averaging of the spectrum – each spectrum displayed is replaced by the next. The acquisition time for each spectrum is set by the user in the Control Panel (see section 9.9.1, page 234).

Spectrum RTD is only possible in a single spectral window range – see section 3.5.6.1, page 50. Extended range RTD measurements (in either multiwindow or auto scanning modes) are not possible. Autofocus and auto exposure modes will not be active for a spectrum RTD measurement, even if they are activated in Acquisition > Options.

The spectrum RTD is useful to adjust the fine focus of the sample to optimise Raman signal, and to monitor whether a sample is degrading or burning. Normally the RTD acquisition time should be kept small in order to allow fast and continuous spectrum read out. Typical values are in the range 0.2s–2s.

To adjust the RTD acquisition time simply type the desired time (in seconds) into the box, and press **<enter ↵ >**.



4.5.2. Detector Image RTD (Real Time Display)

Start a Detector Image RTD acquisition.

The Detector Image Real Time Display (RTD) provides a continuous full image readout of the CCD array detector – it is typically only used for diagnostic and maintenance purposes.

The readout properties are set in Acquisition > RTD – see section 3.5.3, page 33.

4.5.3. Spectrum Acquisition

Start a spectrum acquisition.

The spectrum acquisition will provide full accumulation and averaging of the spectral data, over a user specified range. For more information about setting up the measurement please see information in this manual relating to:

Acquisition options	section 3.5.4, page 34
Auto save	section 3.5.5, page 47
Extended range	section 3.5.6, page 49
Autofocus	section 3.5.8, page 59
Auto exposure	section 3.5.10, page 65

This mode is used to acquire a good quality spectrum of a sample over a user defined spectral range.

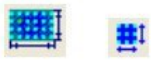
4.5.4. Mapping Acquisition

Start a multidimensional spectral array acquisition, such as time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes.

Mapping acquisition will include full accumulation of the spectral data, over a user specified range. For more information about setting up the measurement please see information in this manual relating to:

Acquisition options	section 3.5.4, page 34
Auto save	section 3.5.5, page 47
Extended range	section 3.5.6, page 49
Autofocus	section 3.5.8, page 59
Auto exposure	section 3.5.10, page 65
Mapping properties	section 4.5.5, page 97

4.5.5. Mapping Properties

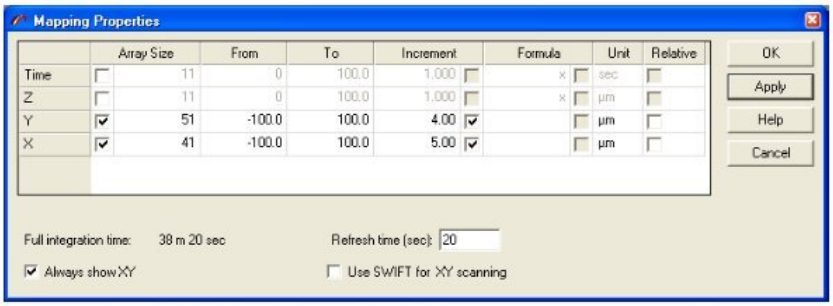


Opens the Mapping Properties dialog window.

The Mapping Properties dialog window allows the properties of a multidimensional spectral array acquisition to be set. This includes measurements such as time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes.

4.5.5.1. Mapping Properties Dialog Window

The Mapping Properties dialog window allows the user to define the acquisition properties of a multidimensional spectral array acquisition. The properties which can be set include number of data points, start and stop positions, and step size. Properties are set independently for each dimension (such as X, Y or Z axes, time, temperature etc).

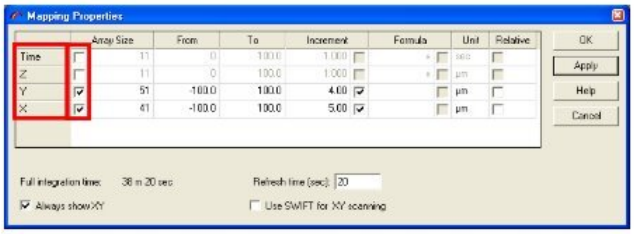


4.5.5.2. Setting the Mapping Properties

The Mapping Properties dialog window displays the following information:

Dimension

The available array dimensions which can be used for multidimensional spectral array acquisitions are shown.



The active dimensions which will be used for the acquisition are shown and ticked. Inactive dimensions which are available but will not be used for the acquisition are shown and unticked.

Dimensions can be added (see section 4.5.5.4, page 100), deleted (section 4.5.5.5, page 101) and moved (see section 4.5.5.3, page 99).

Array Size

The number of data points which will be acquired.

Note that the "Array Size" is directly linked to the "Increment". If the "Increment" is modified, the "Array Size" will be automatically adjusted.

From
The start position/value of the array. The units are as indicated in the "Unit" field. For X and Y dimensions defined on the video image, the "From" value is automatically filled in.

To
The end position/value of the array. The units are as indicated in the "Unit" field. For X and Y dimensions defined on the video image, the "To" value is automatically filled in.

Increment
The step size within the array. The units are as indicated in the "Unit" field. The "Increment" can only be set if the box is ticked. If it is unticked the increment will be automatically calculated according to the "Array Size", "From" and "To" values.

Note that the "Increment" is directly linked to the "Array Size". If the "Array Size" is modified, the "Increment" will be automatically adjusted.

Formula
Set a formula to describe a non-linear array (i.e., an array with non-uniform increment).

A formula can only be set if the "Formula" field is ticked. If "Formula" is active, then the "From", "To", and "Increment" fields will be deactivated and greyed out.

The formula is an arithmetic function of **x**, where **x** is the measurement position within the array, and takes numerical values ranging from 0 (the first position in the array) to [**Array Size – 1**] (the last position in the array).

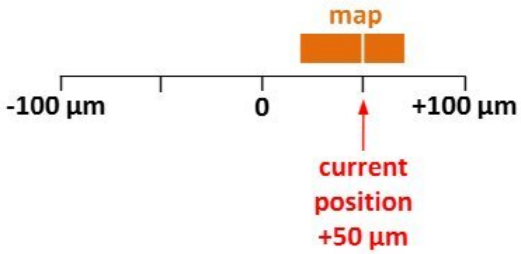
As an example, the formula '(**x** * **x**) + 10' will yield measurements at points 10, 11, 14, 19, 26...

Unit
This field displays the default unit for the dimension. Typically this will be micrometers (µm) for X, Y and Z spatial dimensions, seconds (s) for time, and degrees Celsius (°C) for temperature. If a custom dimension is added to the Mapping Parameters dialog window, the "Unit" field can be set by the user.

Relative
This tick box controls whether the "From" and "To" positions are absolute, or relative to the current position.

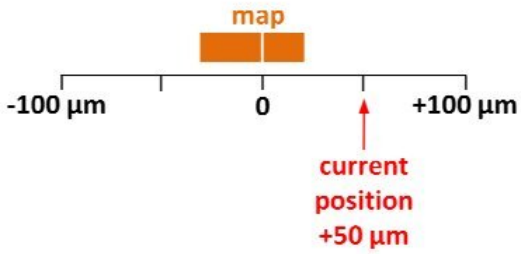
If "Relative" is ticked the "From" and "To" positions are relative to the current position.

For example, if the current X position of the motorized XY sample stage is +50 µm, a line ranging "From"=-30 µm "To"=+20 µm will be acquired from +20 µm to +70 µm (i.e., [+50-30] µm to [+50+20] µm)



If "Relative" is unticked the "From" and "To" positions are absolute.

For example, if the current X position of the motorized XY sample stage is +50 µm, a line set with "From" and "To" values of -30 µm and +20 µm will be acquired from -30 µm to +20 µm.



Always Show XY Parameters

This tick box controls whether the X and Y dimensions are shown at all times. If "Always show XY parameters" is unticked the X and Y dimensions will only be visible when a Video image is open within LabSpec 5.

Use SWIFT for XY Mapping

This tick box controls whether the SWIFT™ ultra-fast mapping mode is used for XY mapping. When "Use SWIFT for XY mapping" is ticked the map will be acquired in the SWIFT™ mode, which is suitable for acquisition times ranging from <1ms through to 1s. When the box is unticked, the map will be acquired in the standard 'point by point' mode, which is suitable for acquisition times greater than 0.5s.

4.5.5.3. Acquisition Order of an Array Measurement

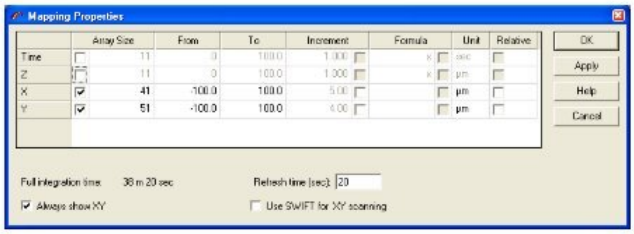
The data acquisition order within a multidimensional spectral array measurement is dependent on the vertical order of the dimensions listed within the Mapping Properties dialog window.

In the first example shown right, "Y" is the top dimension, with "X" listed below it. This means that the array data will be acquired in the following order:

Y_1
$X_1, X_2, X_3, X_4 \dashrightarrow X_n$
Y_2
$X_1, X_2, X_3, X_4 \dashrightarrow X_n$
⋮
Y_n
$X_1, X_2, X_3, X_4 \ldots X_n$

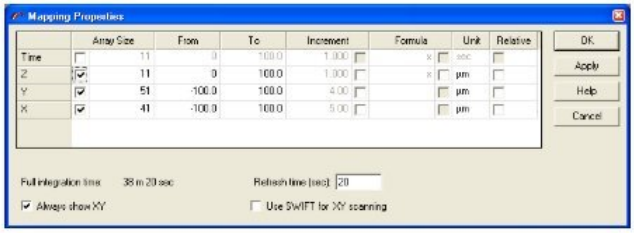
By modifying the order of the dimensions in the list (see section 4.5.5.6, page 101) it is possible to adjust the data acquisition order.

In this case the order has been modified so that "X" is now the top dimension, and "Y" is below it. The acquisition order will now be:



X_1
$Y_1, Y_2, Y_3, Y_4 \dashrightarrow Y_n$
X_2
$Y_1, Y_2, Y_3, Y_4 \dashrightarrow Y_n$
⇣
X_n
$Y_1, Y_2, Y_3, Y_4 \ldots . Y_n$

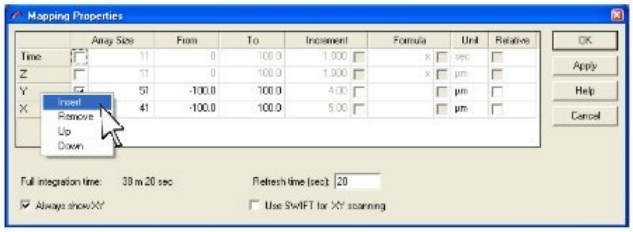
In the second example shown right, "Z" is the top dimension, with "Y" listed below it, and "X" below that. This means that the array data will be acquired in the following order:



Z_1
Y_1
$X_1, X_2, X_3, X_4 \dashrightarrow X_n$
Z_2
Y_2
$X_1, X_2, X_3, X_4 \dashrightarrow X_n$
⇣
Z_n
Y_n
$X_1, X_2, X_3, X_4 \ldots . X_n$

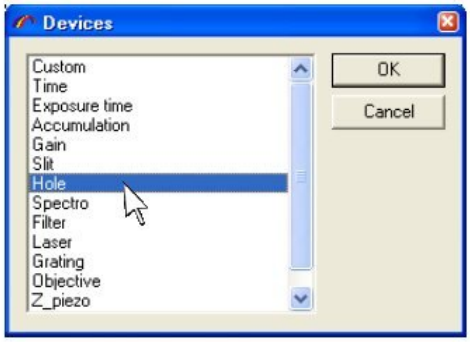
4.5.5.4. Adding New Array Dimensions to the Mapping Properties Dialog Window

To add a new array dimension to the list in the Mapping Properties dialog window, right click on the list of dimensions, and select "Insert".

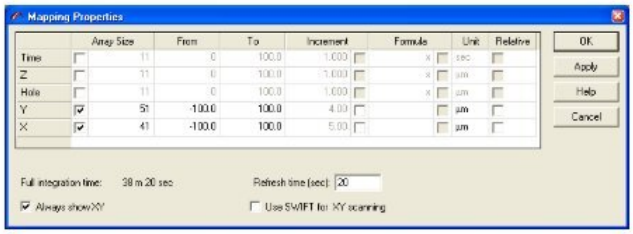


Select the required dimension from the list shown in the Devices dialog window, and click **[OK]**.

Note that all motorized devices correctly configured on your instrument will be shown in this list.

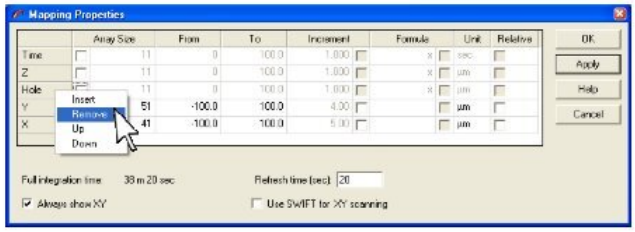


The selected dimension is now displayed in the Mapping Properties dialog window, and is immediately available to be configured within a multidimensional spectral array measurement.



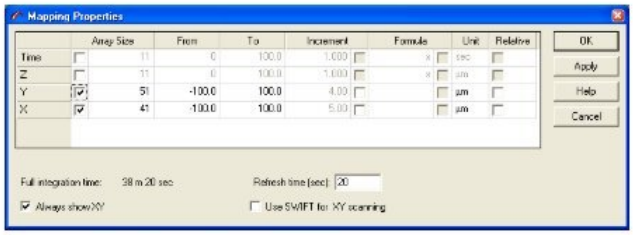
4.5.5.5. Deleting Array Dimensions from the Mapping Properties Dialog Window

To delete a dimension from the list in the Mapping Properties dialog window, right click on the dimension to be deleted, and select “Remove”.



The selected dimension will be removed from the list.

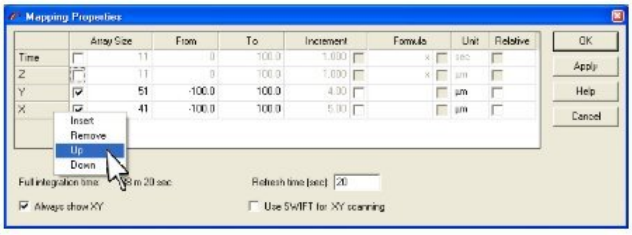
Note that the dimension can be added to the list again by re-inserting it from the list of available devices – see section 4.5.5.4, page 100.



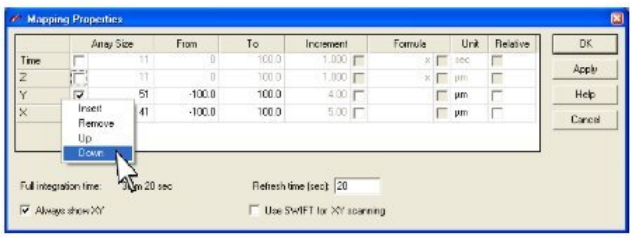
4.5.5.6. Changing the Acquisition Order of an Array Measurement

To adjust the order of dimensions listed in the Mapping Properties dialog window right click on the dimension which is to be moved.

Select “Up” to move the dimension further up in the list.



Select “Down” to move the dimension further down in the list.



4.5.6. Extended Range Acquisition

Opens the Extended Range dialog window (see section 3.5.6, page 49).

The Extended Range dialog window controls the spectral range which will be analysed during a spectrum (/) or multidimensional spectral array (/) acquisition.

4.5.7. Video

Opens the video window and commences live read out of the video camera. On automated systems (such as the XploRA™, LabRAM ARAMIS and automated LabRAM HR) the appropriate optics for using the video camera will also be moved into position.

4.5.7.1. Stopping the Video Read Out

To stop the video read out, click on the **[Stop]** button in the icon bar.

The image currently visible will be captured and displayed as a snapshot. This snapshot image can be saved if desired. See section 3.1.1, page 10, for more information about options for saving video images.

The video read out can be configured using the Video menu – see section 3.6, page 66.

4.5.8. Extended Video

Opens the Extended Video dialog window.

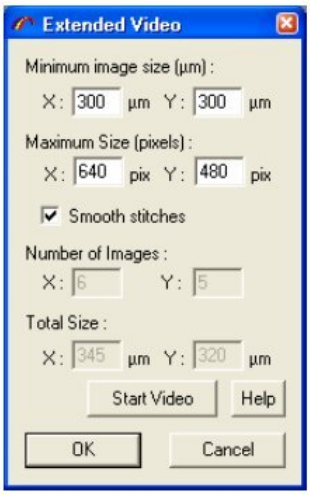
The Extended Video module allows the acquisition of large area video images by montaging single video images.

It is a useful tool to obtain large area images with high magnification objectives. Typically high magnification objectives only have a small field of view (e.g., ~60 µm x 60 µm for a 100x objective), whilst low magnification objectives offer larger views (e.g., ~600 µm x 600 µm for a 10x objective) but at low resolution. With the Extended Video module, the high resolution capability of high magnification objectives can be used without limit over the area which can be viewed.

This module is only available for systems equipped with a motorized XY sample stage.

4.5.8.1. Extended Video Dialog Window

The Extended Video dialog window allows the user to define the acquisition properties of an extended video image acquisition, and provides information about the composition of the final image.



4.5.8.1.1. Setting the Extended Video Properties

The Extended Video dialog window has the following sections:

Minimum Image Size (µm)
The minimum image size required for the extended video image, defined in micrometers (µm) for the X and Y dimensions.

Note that because of the size of individual images and the way they overlap in the montage process the final image may be larger than the dimension set here. The actual size of the extended image to be acquired is shown in the "Total Size" section.

Maximum Size (pixels)
The maximum pixel size of the final image, defined in pixels for the X and Y dimensions.

This control is useful to reduce the file size of the final montaged image. If this image comprises a large number of individual images its raw pixel size will be very large, and will result in a large image file. Reducing this total pixel size to a more appropriate level will result in a small/medium image file.

Note that as the maximum pixel size is decreased both the file size and image quality are reduced; as the maximum pixel size is increased both the file size and image quality are improved. The maximum pixel size possible is limited according to the native pixel size of an individual image and the number of single images contributing to the final extended video image.

Smooth Stitching
When "Smooth Stitching" is ticked, the montage process will include a large overlap between each adjacent image. This is useful to reduce the affect of non-uniform white light illumination on the sample.

Note that when "Smooth Stitching" is used, the number of images used to create the extended video image is increased, and more time will be required to complete the image.

Number of Images
Displays the number of individual images in the X and Y dimensions required to create the specified extended video image.

Total Size
Displays the actual image size (in micrometers, µm) that will be recorded.

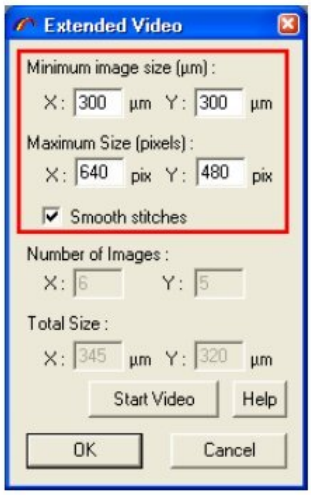
Note that because of the size of individual images and the way they overlap in the montage process the final image may be larger than the dimension set in the "Minimum Image Size (µm)" section.

4.5.8.2. Acquiring an Extended Video Image

To acquire an extended video image click on the Extended Video icon in the icon bar.

Set the required minimum image size (in micrometers, µm) for the X and Y dimensions, and the desired maximum pixel size of the final extended video image.

Ensure the "Smooth Stitching" box is ticked, so that the default smooth montaging mode is used. This mode can be deactivated by unticking the box, but after doing this the final image quality may not be so good.



Click on **[Start Video]** to start the measurement.

The resulting extended video image can be saved if desired. See section 3.1.1, page 10, for more information about options for saving video images.

4.5.9. MultiPoint

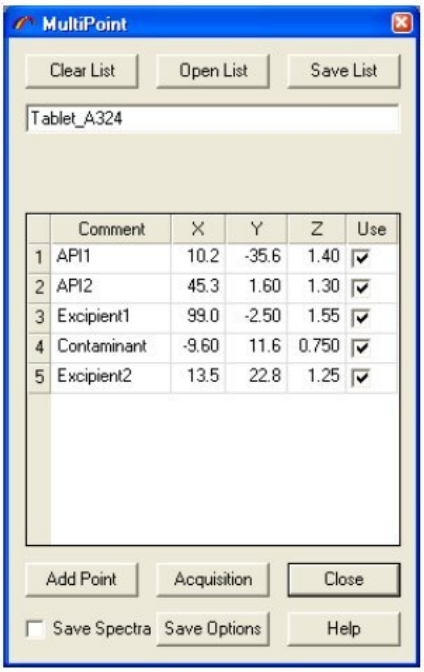
Opens the MultiPoint dialog window.

The MultiPoint module allows automatic acquisition of Raman spectra at multiple positions. Each position can be set in X, Y, and Z dimensions.

This module is only available for systems equipped with motorized XY and Z sample stages.

4.5.9.1. MultiPoint Dialog Window

The MultiPoint dialog window allows the user to define a list of measurement positions, and start the MultiPoint acquisition.



4.5.9.1.1. Using the MultiPoint Dialog Window

The MultiPoint dialog window has the following sections:

Clear List

Click on **[Clear List]** to clear all the points from the currently displayed list.

Open List

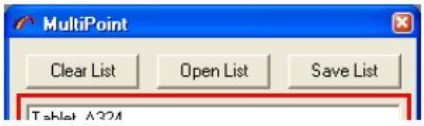
Click on **[Open List]** to open a previously saved list of multipoint positions (in .mpl format). A standard Windows open dialog window will be displayed.

Save List

Click on **[Save List]** to save the currently displayed list of multipoint positions (in .mpl format). A standard Windows save dialog window will be displayed.

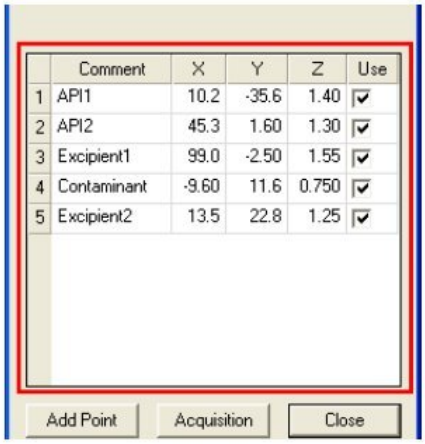
List Description/Name

Type a description or name for the list in the box.



MultiPoint List

Displays the current multipoint positions.



The list contains the following information for each multipoint position:

"Comment" - description or identifier

"X" – X axis stage position (in micrometers, µm)

"Y" – Y axis stage position (in micrometers, µm)

"Z" – Z axis stage position (in micrometers (µm)

"Use" – when ticked this position will be included in the MultiPoint acquisition. When unticked this position will be skipped during the MultiPoint acquisition.

Add Point

Click on **[Add Point]** to add the current XYZ position to the MultiPoint list.

Acquisition

Click on **[Acquisition]** to start the MultiPoint acquisition.

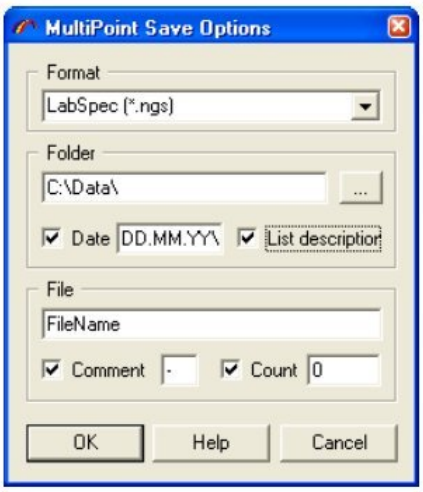
Save Options

Click on **[Save Options]** to set up the autosave procedure for the MultiPoint acquisition. See section 4.5.9.2, page 107, for further information about the save options.

When the "Save Spectra" box is ticked spectra will be automatically saved during the MultiPoint acquisition. If the "Save Spectra" box is unticked spectra will not be saved automatically, but will be available to be saved manually at the end of the MultiPoint acquisition.

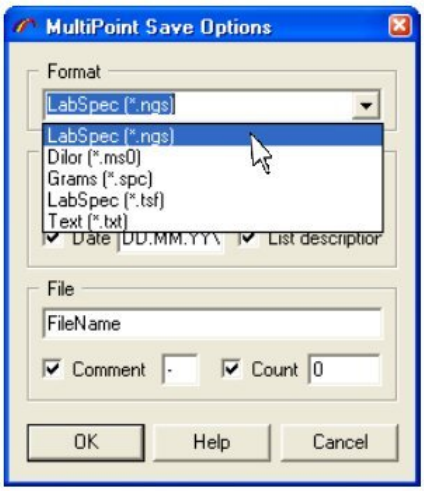
4.5.9.2. Save Options

The MultiPoint Save Options dialog window is used to configure the autosave options for a MultiPoint acquisition.



Format

Select from the "Format" drop down box which file format is to be used to save the MultiPoint spectra. For more information about the available file formats see section 3.1.1.1, page 10.



Folder

Type in the root folder where the data is to be saved, or click on the browse **[...]** button to locate the folder using the standard Windows browse dialog window.

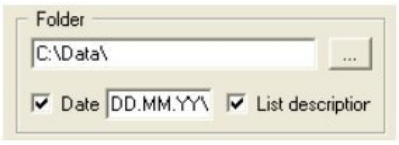
If the "Date" box is ticked, an additional folder will be created in the root folder, labelled DD.MM.YY which indicates the day (DD), month (MM) and year (YY) according to the computer's clock. The date format (e.g., YY.MM.DD or DD.MM.YY) can be adjusted by editing the "Date" text box.

If the "List Description" tick box is ticked an additional folder will be created within the root directory (if "Date" is unticked) or within the "Date" folder (if "Date" is ticked). The folder will be labelled according to the list description/name set in the MultiPoint Parameters dialog window.

Note that if the "\" symbol is removed from the "Folder" text box the date or description label will be appended to the root folder name. If the "\" symbol is removed from the "Date" text box, the description label will be appended to the date folder name.

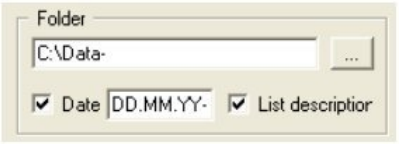
In the example shown right, data will be saved in:

C:\Data\DD.MM.YY\description\



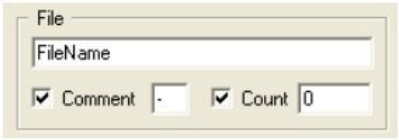
In the example shown right, data will be saved in:

C:\Data-DD.MM.YY-description\



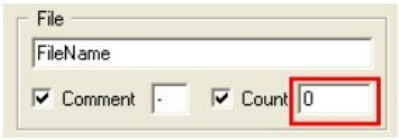
File

Type in the file name which will be used for each MultiPoint spectrum which is to be saved.

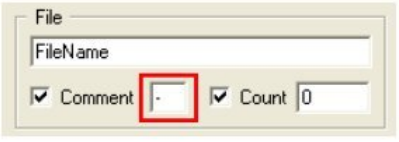


If the "Comment" tick box is ticked the file name will be appended with the comment specified in the MultiPoint Parameters dialog window.

If the "Count" tick box is ticked the file name will be appended with an integer number, starting with the number displayed.



When both "Comment" and "Count" are ticked a seperator can be specified

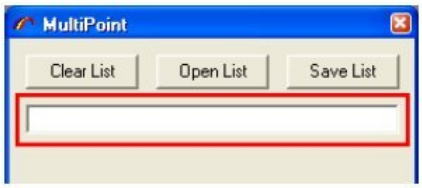


4.5.9.3. Creating a MultiPoint List

To create a MultiPoint list file, open the MultiPoint Parameters dialog window by clicking on the MultiPoint icon.

If there is already a MultiPoint list displayed, click on **[Clear List]** to clear the display. This allows a new list to be created.

Type in a description/name for the list.



Use the video camera and XYZ motorized sample stage to locate a desired position to be added to the MultiPoint list.

Click on **[Add Point]** to add the X, Y and Z coordinate positions (in micrometers, µm) to the MultiPoint list.

Click on the "Comment" field and type in a comment/description for the MultiPoint position.

Repeat the process for additional MultiPoint positions as desired.

When the MultiPoint list is complete, set the save options if desired (by clicking on **[Save Options]**) and tick the "Save Spectra" box to activate the MultiPoint autosave function. See section 4.5.9.2, page 107, for further information about the save options.

The MultiPoint list can be saved (in .mpl format) by clicking on **[Save List]**. A standard Windows save dialog window will be opened.

Set the acquisition parameters in the normal manner. For more information about setting up the measurement please see information in this manual relating to:

Acquisition options	section 3.5.4, page 34
Auto save	section 3.5.5, page 47
Extended range	section 3.5.6, page 49
Autofocus	section 3.5.8, page 59
Auto exposure	section 3.5.10, page 65

Click on **[Acquisition]** to start the MultiPoint acquisition. 

4.5.9.4. Coordinate Positions Used for MultiPoint Acquisition

The coordinate positions used in the MultiPoint list are taken directly from the XYZ Coords section in the Control Panel (see section 9.10, page 236).

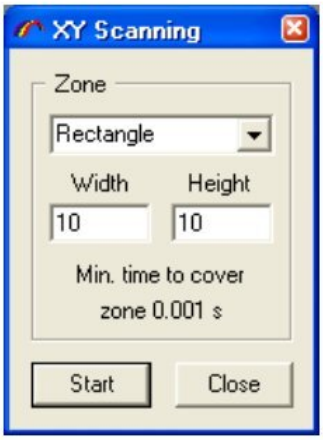
The MultiPoint list is only valid if the sample's position on its holder (e.g., microscope slide) and XY motorized sample stage remains unchanged between defining the list and starting the MultiPoint acquisition.

Similarly, the list is only valid if the XYZ Coord values are not reset to 0 µm in the XYZ Coords section in the Control Panel (see section 9.10, page 236).

4.5.10. DuoScan

Opens the DuoScan™ XY Scanning dialog window.

The XY Scanning dialog window is used to activate the DuoScan™ laser scanning module, and allows the size of the scanning area to be set. The minimum time required for the laser spot to cover the specified area will be displayed.



Zone
Select the type of zone used to define the DuoScan™ scan area from the "Zone" drop down box.

- Rectangle – this allows the user to set a free rectangular area centered around the central laser spot position, according to the dimensions (in micrometers, µm) set in the "Width" and "Height" boxes.
- Mapping Pixel – the scan area is set according to the pixel size specified in the Mapping Properties dialog window (see section 4.5.5, page 97). The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the Mapping Properties dialog window. Note that if SWIFT™ ultra-fast Raman mapping is activated (see section

4.5.5.1, page 97), the "Width" value will be set to 0, because SWIFT™'s continuous scanning in the X direction does not require DuoScan™ scanning in the X (width) direction.

- Video Cursor – the scan area is set according to the dimensions and position of the Rectangular Mapping cursor (see section 5.19, page 187) on the video window. The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the video Rectangular Mapping cursor.

Width* and *Height

Type in the desired DuoScan™ area width and height, in micrometers (µm). These boxes will only be active when the DuoScan™ area zone is set to Rectangle. In other cases the boxes will be greyed out and inactive.

Start

Click on **[Start]** to start the DuoScan™ mirrors scanning the defined area. When DuoScan™ is active, this button will be changed to **[Stop]**.

Stop

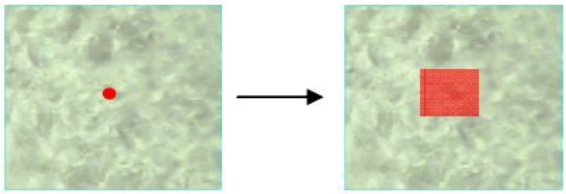
Click on **[Stop]** to stop the DuoScan™ mirrors scanning the defined area. When DuoScan™ is inactive, this button will be changed to **[Start]**.

4.5.10.1. Using DuoScan™

The DuoScan™ scanning mirrors can be used to acquire an average spectrum from an area of the sample (Scanning Mode), or to move the laser spot to specific positions on the sample surface (Point Mode).

4.5.10.1.1. Scanning Mode

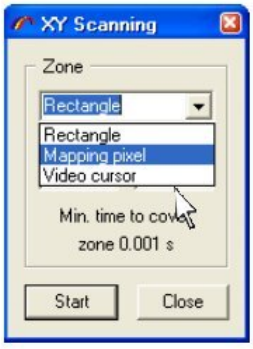
In Scanning Mode DuoScan™ is used to create a laser macro-spot by rapidly scanning the laser spot across a rectangular area. The macro-spot can be used for standard spectrum and multidimensional spectral array data acquisition.



Click on the DuoScan icon to open the DuoScan™ XY Scanning dialog window.

Select the type of zone used to define the area, from the "Zone" drop down box, and set the scan area size in the appropriate way.

- Rectangle – this allows the user to set a free rectangular area centered around the central laser spot position, according to the dimensions (in micrometers, µm) set in the "Width" and "Height" boxes.

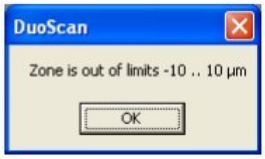


- Mapping Pixel – the scan area is set according to the pixel size specified in the Mapping Properties dialog window (see section 4.5.5, page 97). The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the Mapping Properties dialog window. Note that if SWIFT™ ultra-fast Raman mapping is activated (see section 4.5.5, page 97), the "Width" value will be set to 0, because SWIFT™'s continuous scanning in the X direction does not require DuoScan™ scanning in the X (width) direction.

- Video Cursor – the scan area is set according to the dimensions and position of the Rectangular Mapping cursor (see section 5.19, page 187) on the video window. The "Width" and "Height" boxes will be inactive and greyed out, since the values are taken directly from the video Rectangular Mapping cursor.

Click **[Start]** to activate the DuoScan™ scanning mirrors.

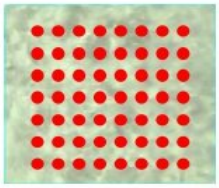
If the scan area has been set greater than the maximum allowable dimensions a warning message will be displayed. This shows the maximum negative and positive displacement possible for the selected objective. The area size set in the "Height" and "Width" boxes, or defined according to the map pixel, or defined by the rectangular video cursor, must not be greater than the total displacement. In the example shown right, the maximum displacement is -10 µm to +10 µm, meaning a total displacement of 20 µm.



The Raman measurement (spectrum or multidimensional spectral array) can now be set up and acquired in the normal way. Remember that the spectrum recorded will be an average spectrum from the scanned area.

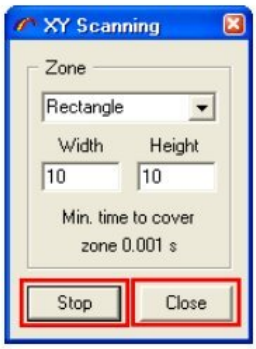
Click **[Stop]** to stop the DuoScan™ scanning mirrors.

4.5.10.1.2. Point Mode



When DuoScan™ is used in Point Mode, it is possible to move the laser spot to any position on the sample surface, within the maximum possible scan displacement. In this mode DuoScan™ can be used as a substitute to the standard motorized stage for point-by-point XY mapping (multidimensional spectral array acquisition) – this function is particularly useful when it is not possible to move the sample underneath the laser beam, or ultra-fine step size is required (down to DuoScan™'s minimum step size of 50 nm).

To use DuoScan™ in Point Mode the scanning mirrors must be static. To confirm this, open the DuoScan™ XY scanning dialog window by clicking on the DuoScan icon.



If the DuoScan™ mirrors are scanning, the **[STOP]** button will be active. Click on **[STOP]** to stop the DuoScan™ scanning, and then click **[CLOSE]** to close the dialog window.

Set the DuoScan™ module as the active XY stage using the 'switch stage' icon in the XYZ Coord section of the Control Panel (see section 9.10.2, page 237).

The laser spot position can now be controlled by inputting XYZ coordinates in the XYZ Coord section of the Control Panel (see section 9.10, page 236), or by acquiring a Raman XY map (multidimensional spectral array) in the standard way. In both cases, the laser spot is now moved across the sample using the DuoScan™ mirrors. The sample does not move.

4.6. Data Processing and Analysis Icons

4.6.1. Spectral ID Search

Launch the 'one click' Spectral ID database search for the active spectrum.

Clicking on this icon will initiate the following processes:

- Export the active spectrum in Grams .spc format
- Open Spectral ID
- Load the exported spectrum into Spectral ID
- Run a full spectrum matching search through the active databases
- Report the list of matching spectra, with match quality scores

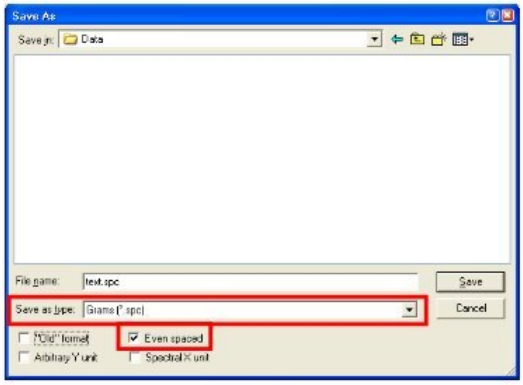
Please see the HORIBA Scientific manual "Using the Spectral ID Database Software with LabSpec 5" and full documentation provided with the Spectral ID database software for more information about Spectral ID and how it can be used and configured.

4.6.1.1. Configuring LabSpec 5 for Correct Spectral ID Searching

The export procedure for the 'one click' Spectral ID search is defined by the options set in the File > Save As dialog window for a Grams .spc file.

To ensure the 'one click' Spectral ID search performs correctly please complete the following set up procedure in LabSpec 5:

- Open a spectrum in any format
- Click on File > Save As
- Select "Grams (.spc)" from the "Save as Type" drop down box
- Tick the box for "Even spaced"
- Click **[Cancel]**



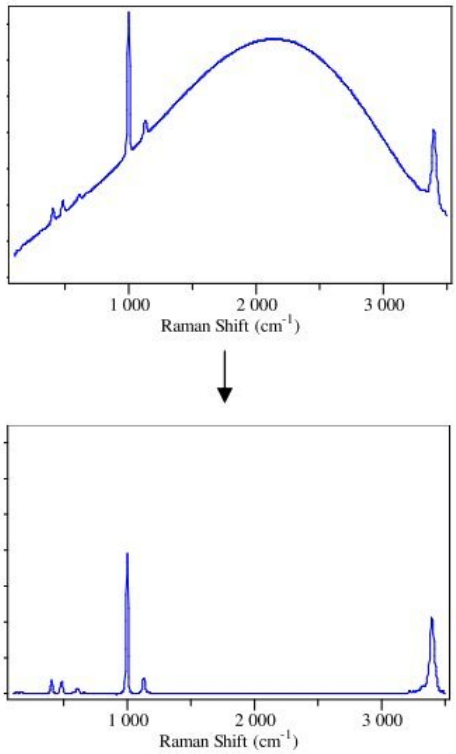
If the "Even spaced" box is not ticked, the Spectral ID search will not be performed correctly, and spurious results may be displayed.

4.6.2. Baseline Correction

Opens the Baseline dialog window.

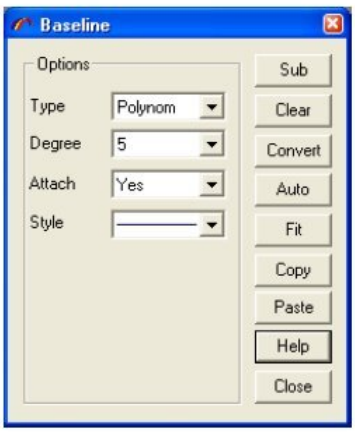
Baseline correction allows a high background in a spectrum to be subtracted, to yield a spectrum with a flat, zero baseline. The correction can be applied to a single spectrum or a multidimensional spectral array of spectra (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes).

The example shown right illustrates a spectrum before and after baseline correction.



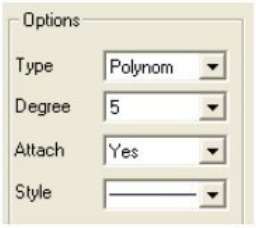
4.6.2.1. Baseline Dialog Window

The Baseline dialog window allows the baseline correction to be configured and performed.



Options

Set the parameters for the baseline. Please see section 4.6.2.1.1, page 117, for information about the individual parameters and their affect on the baseline correction.



Sub

Click on **[Sub]** to subtract the current baseline from the active spectrum.

Clear

Click on **[Clear]** to clear the current baseline from the active spectrum.

Convert

Click on **[Convert]** to convert the active spectrum to the displayed baseline curve. This function is useful to save a baseline curve in a standard spectrum file format.

Note that the active spectrum will be overwritten by the baseline curve. Make sure that the file is saved with a different name to ensure the original spectrum data is not permanently overwritten and lost.

Auto

Click on **[Auto]** to automatically fit a baseline to the active spectrum (based on the parameters set in the "Options" section) and subtract it.

Fit

Click on **[Fit]** to automatically fit a baseline to the active spectrum (based on the parameters set in the "Options" section). The best fit baseline curve will be displayed on the spectrum. It can be subtracted by clicking on **[Sub]**.

Copy

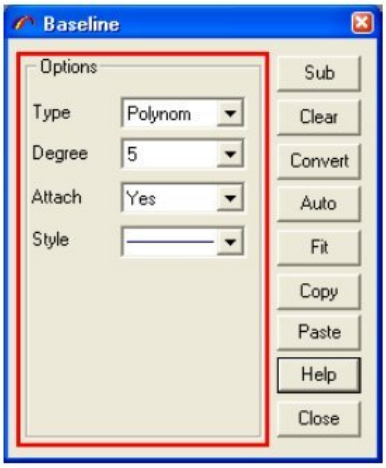
Click on **[Copy]** to copy the displayed baseline curve to the baseline clipboard.

Paste

Click on **[Paste]** to paste a baseline curve from the baseline clipboard onto the active spectrum.

4.6.2.1.1. Baseline Correction Options

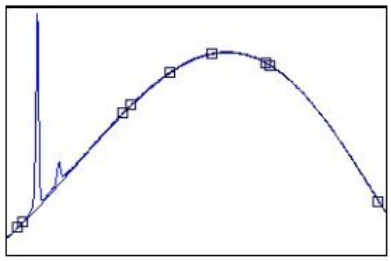
The Baseline options control the parameters of the baseline that will be used for baseline correction.



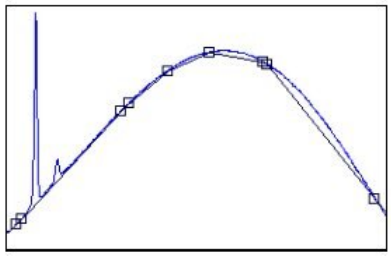
Type

Select the algorithm used to define the baseline curve.

- Polynom: fits a polynomial curve through the baseline points set on the spectrum. The degree of the polynomial is set in the "Degree" drop down box.



- Lines: fits a straight line between baseline points set on the spectrum.



Degree

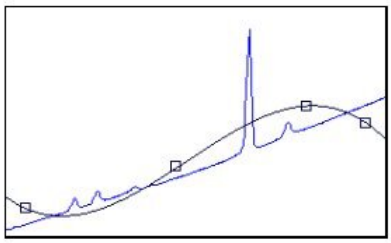
Select the degree of polynomial equation used to create the baseline curve – this option is only used when the baseline "Type" is set to "Polynom".

In general the higher the degree the more adaptable the baseline curve will be to strangely shaped, non-uniform backgrounds.

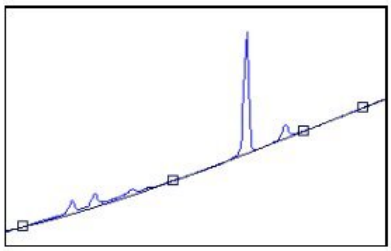
Attach

Select the "Attach" mode for manual definition of baseline points used to define the baseline curve.

- No: baseline points are set at the exact intensity and spectral position (cm^{-1} or nm) specified by the user.



- Yes: baseline points are forced to sit on the spectrum, at the spectral position (cm^{-1} or nm) specified by the user.



The "Attach" mode is particularly important when defining a baseline to be used for correction of multidimensional spectral array data. In this case, "Attach" should be set to "Yes" to ensure that the baseline points will adapt to the varying intensities of the spectra within the multidimensional spectral array.

Style

Set the display style of the baseline curve. The colour, width and line style of the baseline curve can be set using the "Style" drop down box.

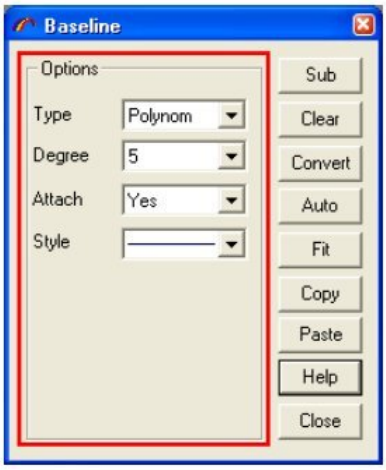
4.6.2.2. Setting a Baseline for Baseline Correction of a Single Spectrum

Select the spectrum which is to be baseline corrected.

Click on the Baseline icon to open the Baseline dialog window.

Select the baseline parameters within the "Options" section. See section 4.6.2.1.1, page 117, for full details about the Baseline Correction Options.

The options can be modified at any point during the process of creating the baseline – the displayed baseline will immediately update.



Click on **[Fit]** to fit a baseline curve to the spectrum.

Baseline points can be manually inserted, adjusted and removed by using the "Add baseline points" icon (see section 5.14, page 180) and "Remove baseline points" icon (see section 5.15, page 181) in the Graphical Manipulation Toolbar.

- Click on the "Add baseline points" icon.

The cursor will change from the mouse cursor to the Add Baseline Points cursor.

Left click on the spectrum to add a baseline point to the displayed baseline curve. If there is no baseline curve on the spectrum the first left mouse click in this mode will create the baseline.

Hover the cursor over an existing baseline point. When the cursor changes from the Add Baseline Points cursor to the Adjust Baseline Points cursor, left click to drag the baseline point to a new position.

- Click on the "Remove baseline points" icon.

Hover the cursor over an existing baseline point. When the cursor changes from the mouse cursor to the Remove Baseline Points cursor, left click to delete

the baseline point from the baseline curve.

The entire baseline can be cleared by clicking on **[Clear]**.

When the baseline curve is completed click **[Sub]** to subtract it from the spectrum.

4.6.2.3. Setting a Baseline for Baseline Correction of a Multidimensional Spectral Array

The procedure as outlined above (section 4.6.2.2, page 118) should be followed, but the baseline curve should be created on the SpIm (spectral image) window.

In general it is useful to have the "Attach" mode set to "Yes" when applying a baseline to the SpIm window, since this ensures the baseline points can adapt to the varying intensities typical in a multidimensional spectral array.

Individual spectra within a multidimensional spectral array can be baseline corrected by applying the correction to the individual spectrum displayed in the Point window.

When the baseline has been subtracted, ensure the corrected spectrum is re-inserted into the spectral array by clicking on the 'blue arrow' icon displayed in the point window, or click **[Correct]** in the Map Analysis dialog window (see section 4.6.9, page 147). This must be done before the map/profile cursor is moved.

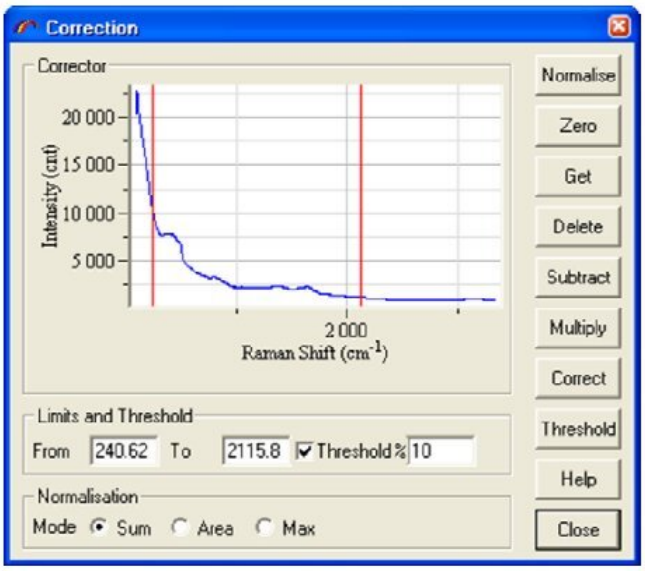
4.6.3. Spectral Normalization and Correction

Opens the Correction dialog window.

The correction functions allow spectra to be normalized and zeroed, and for a substrate/contaminant spectrum to be automatically subtracted.

4.6.3.1. Correction Dialog Window

The Correction dialog window allows the user to apply spectral normalization and correction to both single spectra, and multidimensional spectral arrays.



Normalize

Click on **[Normalize]** to normalize each spectrum so that the total area, sum or maximum signal of the spectrum is 100. If the "Limits" tick box is ticked, the normalization will only be applied within the displayed limits. In this case, the spectrum will be normalized so that the area, sum, or maximum signal within the limits is 100. The limits can be adjusted by typing values in the "Limits" boxes, or adjusting the cursor positions in the "Corrector" window.

The normalization mode can be selected from the "Normalization" section of the dialog window.

Zero

Click on **[Zero]** to automatically subtract a constant intensity value from the active spectrum or multidimensional spectral array, so that the lowest intensity pixel is at zero. When applied to a multidimensional spectral array, each spectrum in the array is zeroed independently.

Get

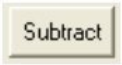
Click on **[Get]** to load the active spectrum in the main LabSpec 5 graphical user interface (GUI) into the "Corrector" window.

Delete

Click on **[Delete]** to clear the "Corrector" window, and remove the corrector spectrum displayed there.

Subtract

Click on **[Subtract]** to subtract the corrector spectrum (displayed in the "Corrector" window) from the active spectrum or multidimensional spectral array.



Multiply

Click on **[Multiply]** to multiply the active spectrum or multidimensional spectral array by the corrector spectrum (displayed in the "Corrector" window).

Correct

Click on **[Correct]** to correct the active spectrum or multidimensional spectral array for the contribution of the corrector spectrum (displayed in the "Corrector" window). The intensity of the corrector spectrum will be automatically adjusted before subtraction to best fit the spectrum.

If the "Limits" tick box is ticked the corrector intensity adjustment will be calculated only within the displayed limits. The limits can be adjusted by typing values in the "Limits" boxes, or adjusting the cursor positions in the "Corrector" window.

This function is useful if a substrate, diluent or contaminant spectrum needs to be removed from a sample spectrum.

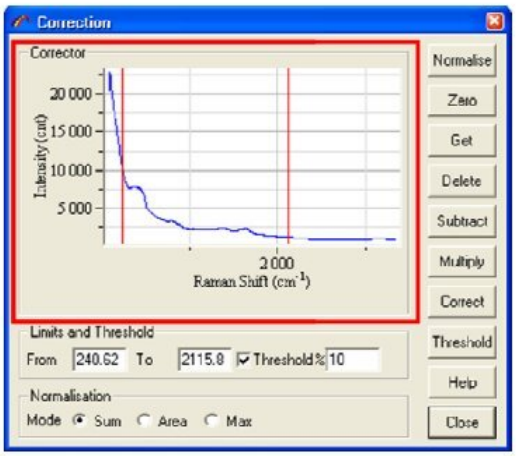
Threshold

Click on **[Threshold]** to threshold the active multidimensional spectral array. The threshold function will be applied to any spectra within the array which has a maximum intensity (relative to the most intensity spectrum in the array) less than the displayed threshold value set in the "Limits and Threshold" section.

The threshold function will convert the spectrum to have zero intensity throughout its spectral range.

Corrector Window

The current corrector spectrum will be displayed in the Corrector window. Click on **[Get]** to load the active spectrum in the main LabSpec 5 graphical user interface (GUI) into the "Corrector" window. Click on **[Delete]** to clear the "Corrector" window, and remove the corrector spectrum displayed there.



The spectrum in the Corrector window can be manipulated using the standard Graphical Manipulation Toolbar icons – see section 5, page 164.

If the cursors are not visible in the Corrector window click on the Center Cursors icon in the Icon bar.

Limits and Threshold

Set the limits for Normalize and Correct functions ticking the "Limits" tick box, and typing the "From" (minimum) and "To" (maximum) limit values.

The limits can also be set by adjusting the position of the cursors in the Corrector window. As the cursors are moved the "From" and "To" values shown in the "Limits and Threshold" section are continuously updated.

Set the threshold value by typing in the desired level (in percent, %) in the "Threshold %" box.

Normalization

Select the normalization mode for the Normalize function.

- {Sum} – normalize so that the sum of the spectrum intensity is 100
- {Area} – normalize so that the area of the spectrum is 100
- {Max} – normalize to the maximum intensity point in the spectrum.

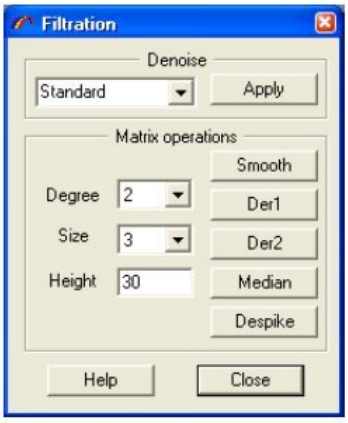
4.6.4. Smoothing

Opens the Filtration dialog window.

The smoothing or filtration functions allow spectra to be smoothed, converted to first and second derivative functions, or despiked. Typically these functions allow spectral quality to be improved after acquisition.

4.6.4.1. Filtration Dialog Window

The Filtration dialog window allows the smoothing and processing functions to be configured and performed on both single spectra, and multidimensional spectral arrays.

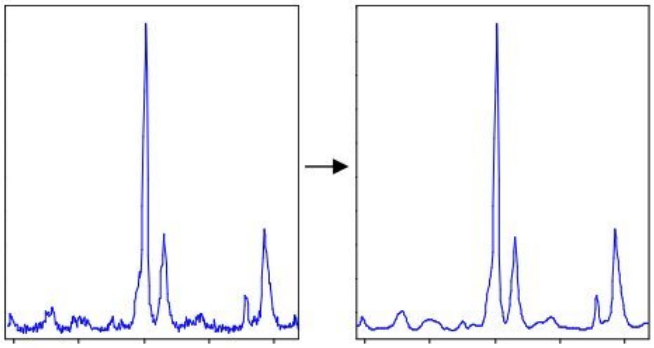


4.6.4.1.1. Denoise

The Denoise function is a unique noise reduction algorithm which can be used to significantly enhance spectrum quality without losing subtle spectral information.

Standard smoothing functions can result in loss of peak shape and position, and subtle features (such as weak shoulders on a strong band) can be lost. The Denoise function ensures that all this important information is retained, whilst still reducing noise in the spectrum.

The spectra shown right illustrate the effect of the Denoise function.



Two main Denoise algorithms are available from the "Denoise" drop down box:

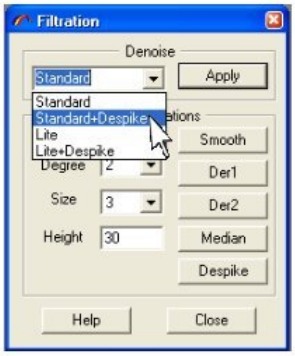
- Standard: recommended for spectra with signal to noise ≥ 20
- Lite: recommended for very noisy spectra with signal to noise ≤ 20

In addition, both algorithms can be used with an integrated Despike function to remove random spikes (also known as cosmic rays). See also section 3.5.4.7, page 37, for more information about other spike filter options in LabSpec 5.

Note that the Denoise function can be automatically applied to all acquired data through the Acquisition > Options dialog window – see section 3.5.4.15, page 46. If a spectrum has had the Denoise function automatically applied through Acquisition > Options, it cannot have the function applied again through the Filtration dialog window.

Using the Denoise Function

Select the desired Denoise algorithm from the "Denoise" drop down box.



Click **[Apply]** to apply the Denoise algorithm to the active spectrum.

4.6.4.1.2. Matrix Operations

The functions described below require the "Degree", "Size", and "Height" values to be set in the drop down boxes, as described in the text.

Smooth

Click on **[Smooth]** to apply a Savitsky-Golay smoothing function to the active spectrum.

Savitsky-Golay smoothing fits a polynomial function of a specified "Degree" through a range ("Size") of adjacent pixels, and replaces those pixels with the polynomial curve. The window where this operation is applied is moved across the entire spectrum. The "Degree" and "Size" must be set to an appropriate level.

Typically the smaller the "Degree" and the larger "Size" the more significant the smoothing. Note that in some cases smoothing can remove or alter

features in a spectrum. Smoothing should be used with care.

Der1

Click on **[Der1]** to convert the active spectrum to its first derivative function. At each pixel position the derivative is calculated using a defined range ("Size") of pixels either side of it.

Der2

Click on **[Der2]** to convert the active spectrum to its second derivative function. At each pixel position the second derivative is calculated using a defined range ("Size") of pixels either side of it.

Median

Click on **[Median]** to apply a non-linear median smoothing function to the active spectrum.

Median smoothing replaces a spectrum pixel intensity value by the median of intensity values within a defined range ("Size") either side of it. This replacement process is repeated for all pixels in the spectrum.

Typically the larger the "Size" the more significant the median smoothing. Note that in some cases median smoothing can remove or alter features in a spectrum. Median smoothing should be used with care.

Despike

Click on **[Despike]** to remove random spikes (also known as cosmic rays) from a spectrum.

A spike is calculated as a pixel which has an intensity greater than the average spectrum intensity + "Height".

The Despike function removes the spike and replaces it with a weighted average of the surrounding pixels.

Note that in some cases the Despike function can remove or alter features in a spectrum, particularly when the spectrum is comprised of very sharp peaks. Despike should be used with care.

Please also see section 3.5.4.7, page 37, for more information about other spike filter options in LabSpec 5.

4.6.5. Fourier Transform

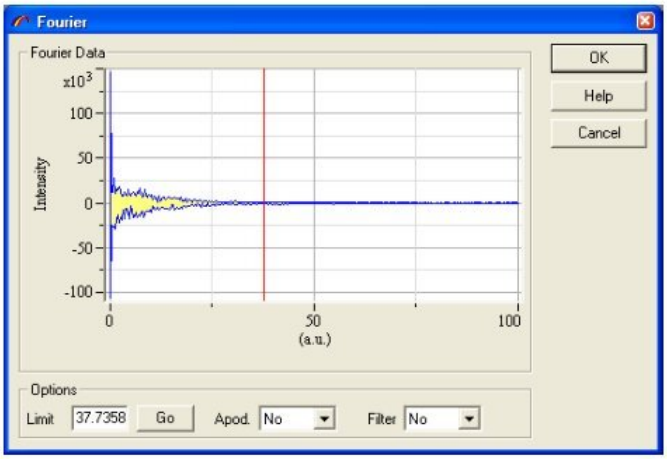
Opens the Fourier Transform dialog window.

The Fourier Transform function allows smoothing of a spectrum based on direct Fourier data transformation, applying filter and apodization functions. The spectrum is converted into its real and imaginary Fourier functions, which essentially represents the spectrum as a combination of wave patterns of varying frequency. Smoothing can be applied by removing high frequency contribution (corresponding to noise) and leaving medium and low frequency contribution (corresponding to Raman peaks).

The Fourier Transform smoothing function can be performed on both single spectra, and multidimensional spectral arrays.

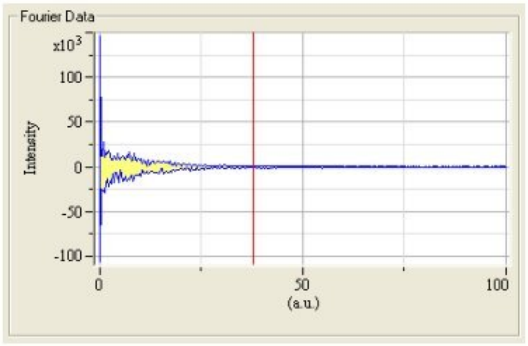
4.6.5.1. Fourier Transform Dialog Window

The Fourier Transform dialog window displays the Fourier functions of the active spectrum, and provides Apodization and Filter options for the transformation.



Fourier Data

The Fourier Data window displays the real and imaginary Fourier functions of the active spectrum.



The red cursor can be used to set the high frequency limit – frequencies above the limit position will be removed from the spectrum.

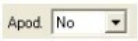
Typically the lower the cursor position the more smoothing is applied. At position 0 the spectrum is fully smoothed (to a flat line). At position 100 the spectrum is fully unsmoothed.

Limit

The Limit allows manual control of the "limit" cursor displayed in the Fourier Data window. Type in the required cursor position value (ranging between 0 and 100) and click on **[Go]**.

Apod.

Select the type of apodization to be used in the Fourier transformation from the "Apod." drop down box. The apodization reaches zero at the Limit position.



Four modes are available:

- No: no apodization
- Line: linear apodization function
- Sqrt: parabolic apodization function
- Cos: cosine apodization function

Filter

Select whether a filter will be used for the Fourier transformation, using the "Filter" drop down box.

Two modes are available:

- No: no filter
- Traffic: traffic filter

OK

Click **[OK]** to permanently apply the smoothing to the active spectrum, and close the Fourier Transform dialog window.

Cancel

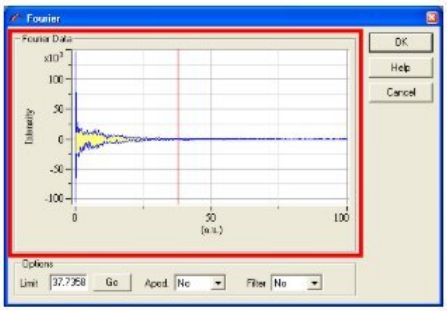
Click **[Cancel]** to close the Fourier Transform without applying the smoothing. The original spectrum will be left unchanged.

4.6.5.2. Using the Fourier Tranform Function to Smooth a Spectrum

Select a spectrum to be smoothed.

Open the Fourier Transform dialog window by clicking on the Fourier Transform icon in the Icon Bar.

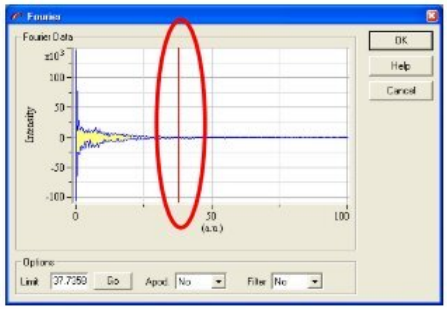
The real and imaginary Fourier functions of the active spectrum are displayed in the Fourier Data window.



Select the Apodization and Filter functions to be used from the "Apod." and "Filter" drop down boxes.

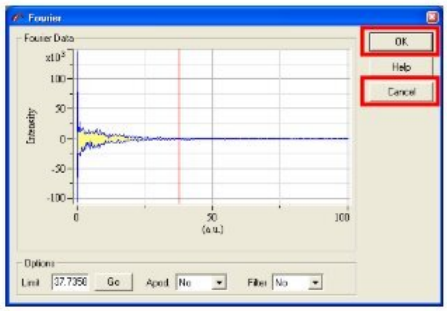
Set the limit for high frequency contribution which will be removed from the spectrum. The spectrum is continuously updated allowing the degree of smoothing to be monitored. The limit can be set in two ways:

- Click and drag the red cursor in the Fourier Data window.

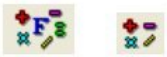


- Type in the required limit into the "Limit" box, and click **[Go]**.

When the desired smoothing is achieved, click **[OK]** to permanently apply the smoothing to the active spectrum and close the Fourier Transform dialog window. Alternatively click **[Cancel]** to close the dialog window without applying any smoothing to the spectrum. The spectrum will be left unchanged.



4.6.6. Math



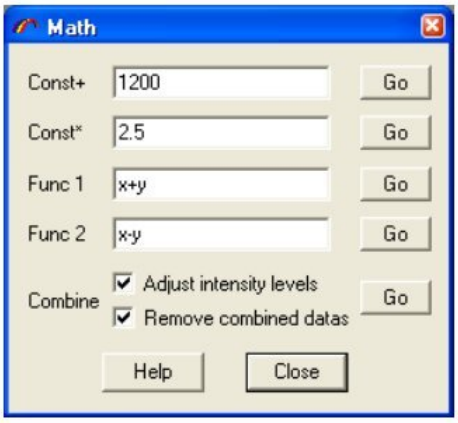
Opens the Math dialog window.

Arithmetic functions can be applied to the intensity values of an active spectrum. Additionally, the Extended Range spectral acquisition "Combine data" function can be applied post-acquisition through the Math dialog window.

The Math functions can be performed on both single spectra, and multidimensional spectral arrays.

4.6.6.1. Math Dialog Window

The Math dialog window contains text input boxes so that arithmetic functions can be created, and then applied to the active data.

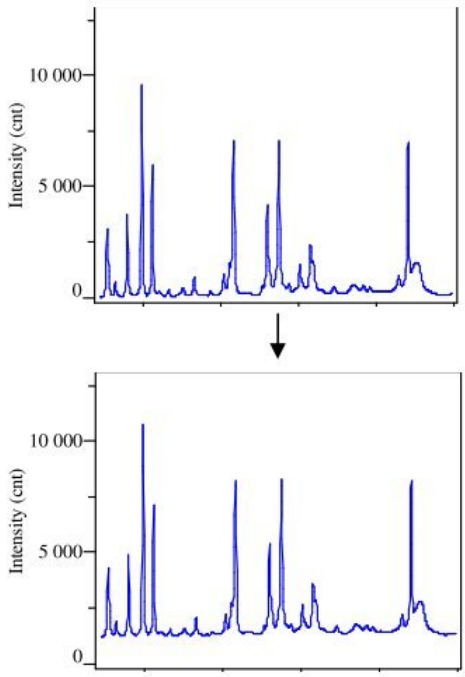


Const+

The "Const+" function adds a constant intensity value to all pixels in the active spectrum.

Type in the desired constant, and click on the adjacent **[Go]** button to apply the function to the active spectrum. A positive constant will be added to the spectrum intensity values; a negative constant will be subtracted from the spectrum intensity values.

The example right shows the result of applying "Const+"=1200 to a spectrum.



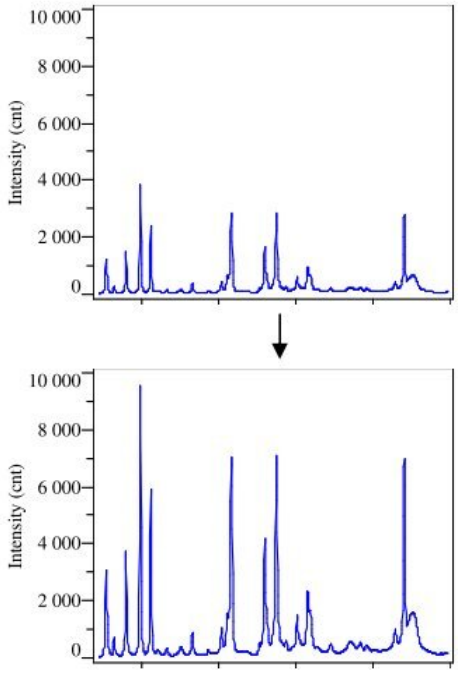
The "Const+" function can also be applied using the "Add Constant" icon in the Graphical Manipulation toolbar – see section 5.8, page 170.

Const*

The "Const*" function multiplies all pixel intensity values in the active spectrum by a constant value.

Type in the desired constant, and click on the adjacent **[Go]** button to apply the function to the active spectrum. A constant greater than zero will increase the spectrum intensity; whilst a constant less than zero will decrease the spectrum intensity.

The example right shows the result of applying "Const*"=2.5 to a spectrum.



The "Const*" function can also be applied using the "Multiply by Constant" icon in the Graphical Manipulation toolbar – see section 5.9, page 171.

Func 1 and Func 2

The "Func 1" and "Func 2" sections allow arithmetic functions to be created by the user, and applied to the active spectrum.

The terminology used for these functions are as follows:

- **x** and **y** refer to the intensity values of spectra which are open in LabSpec 5. **x** is the active data file, and **y** is the other data file open in LabSpec 5. In the event that there is more than one spectrum which could be used for **y** a message box will ask for the **y** spectrum to be chosen from a list.
- **a**, **b** and **c** refer to the values of the first, second and third axes of the active data file.
- Standard arithmetic functions are also possible, including **+**, **-**, *, /, **^**, **exp**, **log**, **sin**, **asin**, **cos**, **acos**, **tan**, **atan**, **abs**, **sqrt** etc.

As an example, if "Func 1" = **x** + **y**, **x** refers to the intensity value at each pixel of the active spectrum, and **y** refers to the intensity value at each pixel of another open spectrum. Assume the two spectra have values as follows (where **a** represents the spectral axis):

a →	1	2	3	4	5	6	7	8	...
x →	10	12	15	25	22	13	10	7	...
y →	1	2	3	2	1	1	4	3	...

applying the function **x** + **y** to this data will result in the following values:

a →	1	2	3	4	5	6	7	8	...
x →	**11**	**14**	**18**	**27**	**23**	**14**	**14**	**10**	**...**
y →	1	2	3	2	1	1	4	3	...

As another example, if "Func 1" = **x** + 2***a**, **x** refers to the intensity value of each pixel of the active spectrum, and **a** refers to the first axis (i.e., the spectral axis, with units Raman shift, cm^{-1}) value of each pixel. Assume the data has the following values:

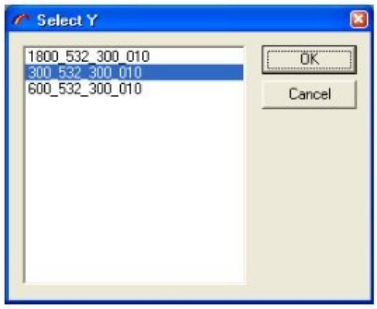
a →	1	2	3	4	5	6	7	8	...
x →	10	12	15	25	22	13	10	7	...

applying the function **x** + 2***a** to this data will result in the following values:

a →	1	2	3	4	5	6	7	8	...
x →	**12**	**16**	**21**	**33**	**32**	**25**	**24**	**23**	**...**

Type in the desired arithmetic function for either "Func 1" or "Func 2", select the appropriate active spectrum (corresponding to **x** in the function), and click on the adjacent **[Go]** button.

If there are multiple options for the spectrum **y**, a "Select Y" message box will ask for the desired spectrum to be selected. Click on the desired spectrum and then click **[OK]** to complete the arithmetic procedure.

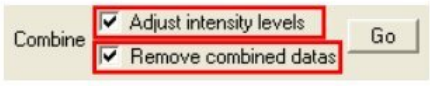


Combine

The "Combine" function allows individual spectral windows in an Extended Range spectrum acquisition to be glued together to yield a single spectrum. This process can also be applied automatically during an Extended Range acquisition (see section 3.5.6, page 49).

Equally, the "Combine" function can be used to create an average spectrum from all open spectra.

Set the options as desired:

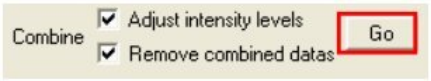


- Adjust intensity levels: if this box is ticked the baselines of the individual spectral windows will be adjusted prior to gluing,

to yield a seamless final spectrum. See section 3.5.6.3, page 53, for full information about this mode.

- Remove combined datas: if this box is ticked the individual spectra or spectral windows will be deleted after combination, leaving only the single combined spectrum on screen.

Click on the adjacent **[Go]** button to apply the "Combine" process.



4.6.7. Peak Searching and Fitting

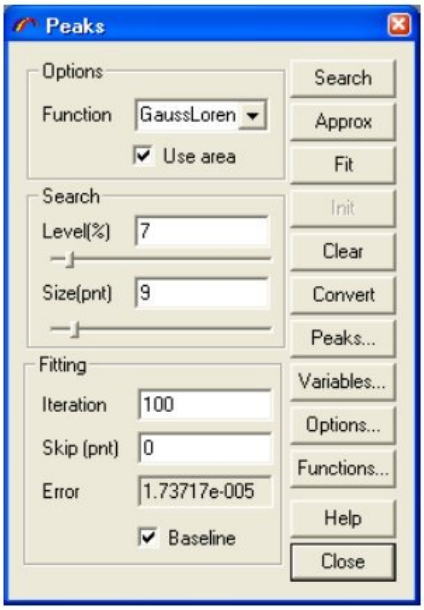
Opens the Peaks dialog window.

The Peak Searching and Fitting module allows peaks in a spectrum to be automatically labelled by their position, and full peak fitting can be carried out to fully characterise peak parameters such as position, amplitude, full width at half maximum height (FWHM) and area. Overlapping peaks can be fully deconvoluted through the peak fitting routine.

The Peak Searching and Fitting functions can be performed on both single spectra, and multidimensional spectral arrays.

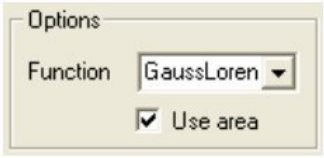
4.6.7.1. Peaks Dialog Window

The Peaks dialog window allows the peak searching, labelling and fitting processes to be configured and applied, and displays the peak parameters after fitting.



Peak Options

Select the appropriate function to be used for peak fitting from the "Function" drop down box. Three default functions are provided:



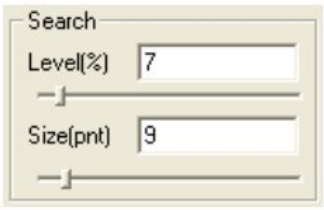
- Gaussian
- Lorentzian
- Mixed Gaussian-Lorentzian

Other functions can be defined by clicking on **[Functions...]** in the Peaks dialog window – see 4.6.7.5, page 144, for full information.

If "Use area" is ticked the peak fitting routines will also calculate the area of the peak(s). When "Use area" is unticked only the default peak parameters will be calculated. These are peak position, amplitude (i.e., maximum height), full width at half maximum height (FWHM) and (for mixed Gaussian-Lorentzian functions) the degree of Gaussian contribution.

Search Options

Set the parameters used for automatic peak searching and identification. The search routine locates local intensity maxima, and assigns these as peaks.



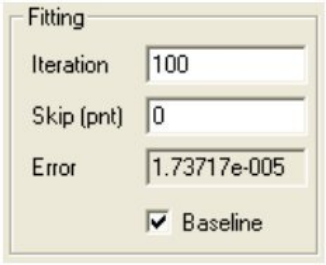
A local maximum must be greater than a certain percentage of the maximum intensity in the whole spectrum. The "Level (%)" parameter defines this percentage of maximum spectral intensity. Typically, as the "Level (%)" is increased, only the most intense peaks will be identified. If low intensity peaks need to be identified "Level (%)" should be reduced.

A local maximum is assumed to exist within a finite number of adjacent data points. The "Size (pnt)" parameter defines this number. Typically, as the "Size (pnt)" is increased, only peaks which are widely separated will be identified. If close lying peaks need to be identified "Size (pnt)" should be reduced.

The "Level (%)" and "Size (pnt)" values can be set by typing a value in the appropriate box, or using the appropriate scroll bar. The peak labelling displayed on the active spectrum will update continuously, so that the result can be monitored.

Fitting Options

Set the parameters used for the peak fitting routine. This routine uses a Levenberg-Marquardt non-linear peak fit algorithm, and iteratively adjusts all peak parameters to minimise the standard error.



The maximum number of iterations can be set by typing an appropriate number in the "Iteration" box. Typically the larger the iteration number the more accurate the final fit result will be, but the longer the process will take.

The algorithm can be set to miss out data points within the spectrum, in order to speed up the process. "Skip (pnt)" is used to define how many points are missed.

- "Skip (pnt)" = 0, all data points are used for the fitting.
- "Skip (pnt)" = 1, every second data point is used for the fitting routine.
- "Skip (pnt)" = 2, every third data point is used for the fitting routine.

Typically as "Skip (pnt)" is increased the fit results will be less accurate, but the process will be faster.

The "Error" box displays the Standard Error between the fit result and the raw data. The smaller the Standard Error the more accurate the fit result.

If the "Baseline" box is ticked the peak fitting routine will additionally fit the specified baseline. The baseline must be specified first, using the Baseline dialog window – see section 4.6.2.1, page 115.

Search

Click on **[Search]** to start the automatic peak searching and identification routine.

Adjust the "Size (pnt)" and "Level (%)" to control the searching and identification procedure – see above for more information about these parameters.

Approx

Click on **[Approx]** to run the peak approximation routine, which can be used to estimate the initial peak parameters prior to fitting. Only the peak position and width parameters are adjusted.

The peak approximation is a useful function to assist in complex peak fitting procedures. Running the peak approximation routine prior to full fitting ensures that the starting parameters are realistic and close to their true values. This reduces the possibility of the peak fitting routine locating an incorrect solution.

Fit

Click on **[Fit]** to run the peak fitting routine, which can be used to calculate peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area.

The peak fitting routine can only be used if peaks have been located in the spectrum. Peaks can be located automatically using the **[Search]** button, or manually by using the "Add peak" icon in the Graphical Manipulation toolbar (see section 5.10, page 172).

Init

Click on **[Init]** to restore peak parameters in the SpIm window of a multidimensional spectral array to the initial values before the peak approximation **[Approx]** or fitting **[Fit]** routines were run.

Clear

Click on **[Clear]** to clear all peaks from the active spectrum.

Convert

Click on **[Convert]** to convert the active spectrum to the sum of the displayed peaks. This function is useful to save a theoretical peak fit solution in a standard spectrum file format.

Note that the active spectrum will be overwritten by the sum of the displayed peaks. Make sure that the file is saved with a different name to ensure the original spectrum data is not permanently overwritten and lost.

Peaks...

Click on **[Peaks...]** to open the Peak Parameters dialog window, to view and manually set peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area values for all peaks labelled on a spectrum.

See section 4.6.7.2, page 138, for more information.

Variables...

Click on **[Variables...]** to open the Peak Variables dialog window, to view and set initial values and maximum/minimum values for variables within the peak fitting routine.

See section 4.6.7.3, page 140 for more information.

Options...

Click on **[Options...]** to open the Peak Options dialog window, to view and set display options for the peak labelling and fitting.

See section 4.6.7.4, page 143, for more information.

Functions...

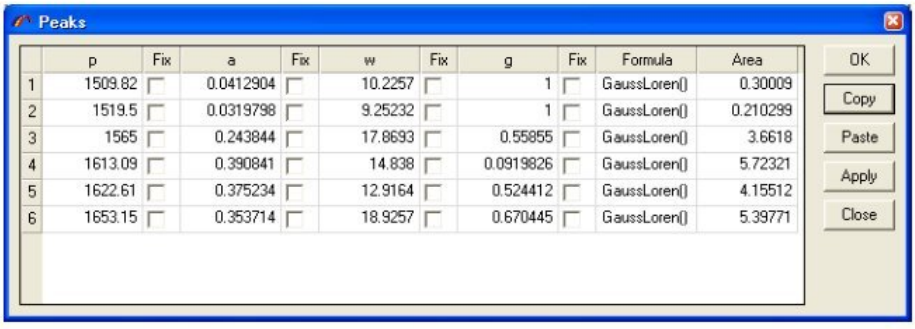
Click on **[Functions...]** to open the Peak Functions dialog window, to view and create user defined peak shape functions.

See page 4.6.7.5, page 144, for more information.

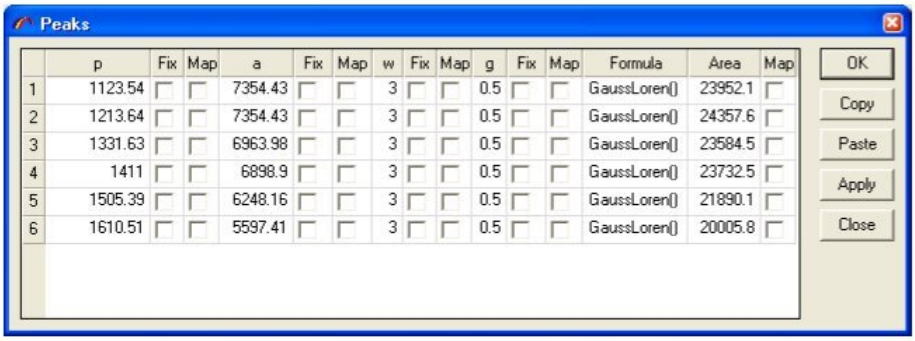
4.6.7.2. Peak Parameters Dialog Window

The Peak Parameters dialog window displays peak position, amplitude, full width at half maximum height (FWHM), Gaussian contribution and area for all peaks labelled on a spectrum. The parameters can be manually adjusted and fixed so that the parameter is not varied during the fitting routine.

The Peak Parameters dialog window for individual spectra (e.g., Spectrum window, or Point window of a multidimensional spectral array) has the following appearance:



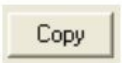
The Peak Parameters dialog window for multidimensional spectral arrays (e.g., the SpIm window of a multidimensional spectral array) has the following appearance:



Each row displays the parameters for a single peak:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s).
- **w** – peak full width at half maximum height (FWHM) in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **g** – Gaussian contribution in a mixed Gaussian-Lorentzian function. The value of Gaussian contribution varies from 0 (no Gaussian contribution, fully Lorentzian) through to 1 (fully Gaussian). The **g** column is only displayed when a mixed Gaussian-Lorentzian function is selected for peak fitting in the main Peaks dialog window (see section 4.6.7.1, page 134).
- **Formula** – the function used for the peak fitting, as selected in the main Peaks dialog window (see section 4.6.7.1, page 134). "Gauss()" = Gaussian, "Loren()" = Lorentzian, "GaussLoren()" = mixed Gaussian-Lorentzian.
- **Area** – the area of the peak, in area units based on the units displayed on the spectrum's X and Y axes. The **Area** column is only displayed if the "Use area" box is ticked in the main Peaks dialog window (see section 4.6.7.1, page 134).
- **Fix** - the "Fix" tick boxes to the right of each of the **p**, **a**, **w** and **g** parameters allows a parameter to be fixed. A fixed parameter will not be varied during the peak fitting routine. When a box is ticked the parameter is fixed. When a box is unticked the parameter will be varied during the fitting routine.
- **Map** – the "Map" tick boxes (which are only displayed for the SpIm window of a multidimensional spectral array) to the right of each of the **p**, **a**, **w**, **g** and **Area** parameters allows a profile or map image to be generated based on the parameter. For example, it is possible to create an image based on peak position, illustrating how the peak position varies across the map area. To display a profile/image based on a peak parameter tick the appropriate box and click **[Apply]**. A new map profile/image will be created. To close the map profile/image, untick the box and click **[Apply]**.

Copy



Click on **[Copy]** to copy the parameters displayed in the Peak Parameters dialog window. Parameters which have been copied can be pasted into other programmes (such as Microsoft Office) or into the Peak Parameters dialog window.

This function can be used to copy peak parameters from one spectrum and paste them to another spectrum (using **[Paste]**).

Paste

Click on **[Paste]** to paste parameters in the Peak Parameters dialog window.

This function can be used to paste peak parameters copied (using **[Copy]**) from one spectrum to another.

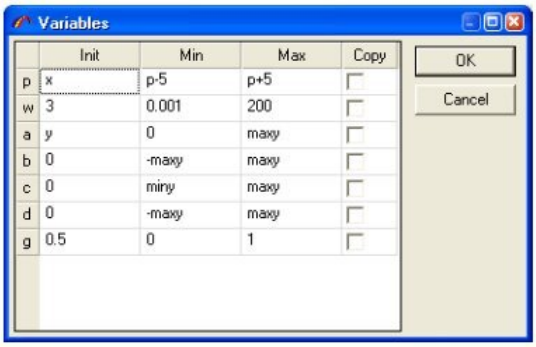
Apply

Click on **[Apply]** to update the peak(s) displayed on the spectrum according to parameters in the Peak Parameters dialog window.

This button must be used when parameters are manually adjusted in the Peak Parameters dialog window. The peaks displayed on the spectrum will not reflect the new parameters until **[Apply]** is clicked.

4.6.7.3. Peak Variables Dialog Window

The Variables dialog window displays the initial parameters used in the peak fitting procedure, and the minimum and maximum values they can take during the fitting procedure. In most cases the default values are suitable for general peak fitting routines, but in specific cases the initial parameters and their minimum and maximum values can be manually adjusted as required.



The Variables dialog shows the initial ("init") values, and minimum ("Min") / maximum ("Max") values for each parameter:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm). In the example shown above, the initial value of **p** is the X axis position ("x") at which the peak is initially located or positioned, and the position can be varied from **p**-5 to **p**+5 during the fitting procedure.
- **w** – peak full width at half maximum height, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm). In the example shown above, the initial value of **w** is 3, and width can be varied from 0.001 to 200 during the fitting procedure.
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s). In the example shown above, the initial value of **a** is the Y axis position ("y") at which the peak is initially located or positioned, and the amplitude can be varied from 0 to the maximum Y axis value ("maxy") during the fitting procedure.

- **b**, **c**, **d** – parameters within the Gaussian/Lorentzian equations. In the example shown above, the initial values of **b**, **c** and **d** are 0, and they can be varied from either the inverse of the maximum Y axis value ("-maxy") or the minimum Y axis value ("miny"), to the maximum Y axis value ("maxy") during the fitting procedure.
- **g** – Gaussian contribution in a mixed Gaussian-Lorentzian function. In the example shown above, the initial value of **g** is 0.5, and the Gaussian contribution can be varied from 0 (no Gaussian contribution, fully Lorentzian) through to 1 (fully Gaussian) during the fitting procedure.

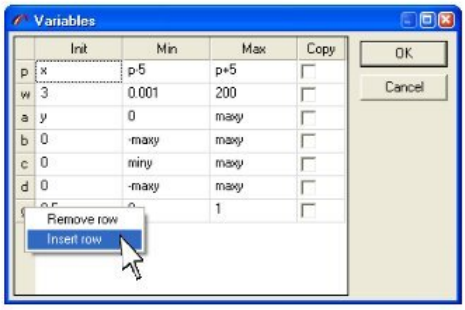
If "Copy" is ticked for a parameter the "min" and "max" values will be displayed individually for each peak in the Peak Parameters dialog window. This allows "min" and "max" parameters to be set individually for each peak. Note that the "Copy" box must be ticked before the peaks are automatically located using **[Search]** or manually located using the "Add peak" icon in the Graphical Manipulation toolbar (see section 5.10, page 172).

4.6.7.3.1. Setting the Init, Min and Max Values in the Variables Dialog Window

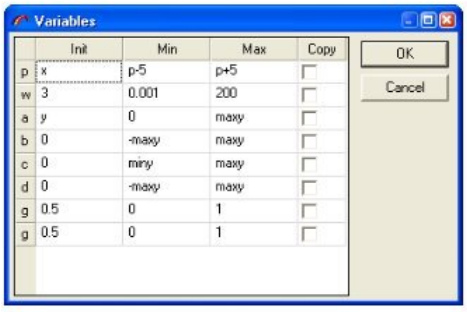
Left click in the desired parameter "init", "min" or "max" box and insert the desired value. Add information for each parameter as required.

4.6.7.3.2. Adding Parameters to the Variables Dialog Window

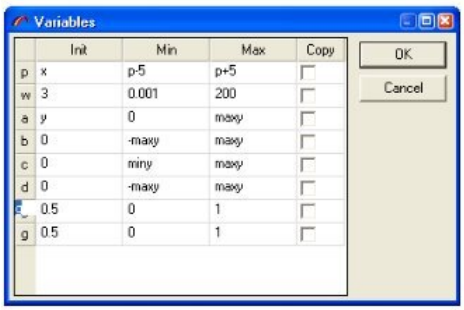
Additional categories can be added by right clicking on one of the parameter name boxes and selecting "Insert row".



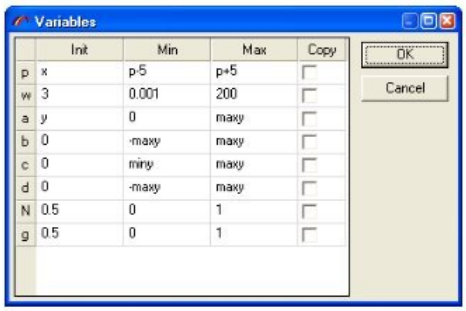
A new row will be inserted above the selected position, and will display the same name and "init", "min" and "max" values as the original parameter.



Double click on the parameter name box to allow the name to be edited. Type in the desired category name.



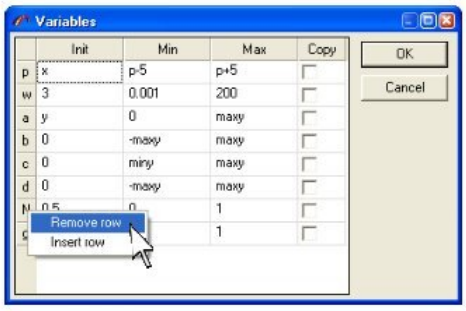
Click on any other parameter name box to register the new name.



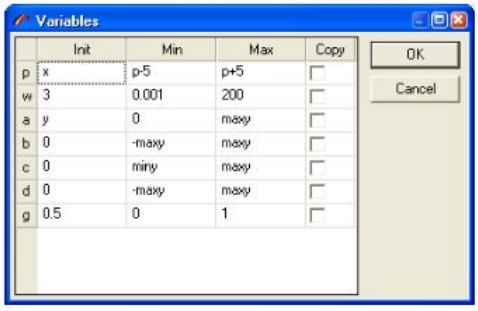
The name given to the new parameter must exactly match the name used in definition of functions in the Peak Functions dalog window (see section 4.6.7.5, page 144). The “init”, “min” and “max” values must be set to appropriate values.

4.6.7.3.3. Deleting Parameters from the Variables Dialog Window

Parameters can be deleted by right clicking on the parameter name boxes which is to be deleted and selecting “Remove row”.

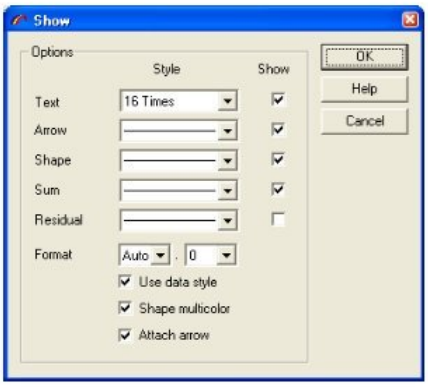


The parameter will be deleted from the Variables dialog window.



4.6.7.4. Peak Options Dialog Window

The Peak Options dialog window allows control of the display options for the peak labelling and fitting.



The following peak labelling and fitting display components can be controlled:

Text

Click on the Text "Style" drop down box to set the font, font style, font size and font color used to display the peak position on the spectrum. If "Show" is ticked the peak position will be displayed on the spectrum. Note that if "Use data style" is ticked the colour will be set to match the spectrum color. If "Use data style" is unticked the colour will be set according to the selection made in the "Style" drop down box.

Arrow

Click on the Arrow "Style" drop down box to set the colour, width and line style for the arrow marker indicating the peak position. If "Show" is ticked the arrow marker will be displayed on the spectrum. Note that if "Use data style" is ticked the colour will be set to match the spectrum color. If "Use data style" is unticked the colour will be set according to the selection made in the "Style" drop down box.

Shape

Click on the Shape "Style" drop down box to set the colour, width and line style for the individual peak shape(s) displayed on the spectrum. If "Show" is ticked the peak shape(s) will be displayed on the spectrum. Note that if "Shape multicolor" is ticked the colour will be automatically selected from a default palette; in this case, when multiple shapes are displayed on a single spectrum each shape will be a different color. If "Shape multicolor" is unticked the colour will be set according to the selection made in the "Style" drop down box; in this case, when multiple shapes are displayed on a single spectrum each shape will be the same colour.

Sum

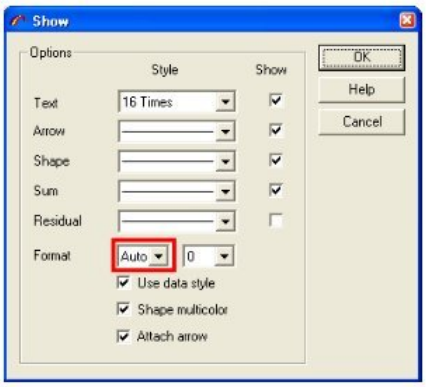
Click on the Sum "Style" drop down box to set the colour, width and line style for the sum spectrum (i.e., the combination spectrum created by summing all the peak shapes displayed). If "Show" is ticked the sum spectrum will be displayed on the spectrum.

Residual

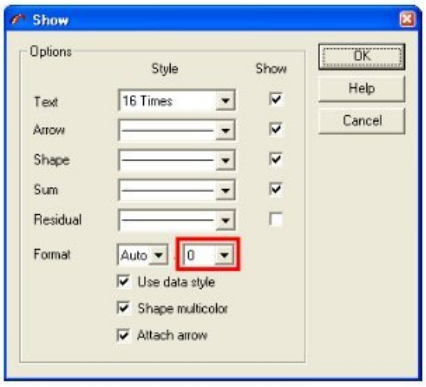
Click on the Residual "Style" drop down box to set the colour, width and line style for the residual spectrum (i.e., the difference between the sum spectrum and the raw data). If "Show" is ticked the residual spectrum will be displayed on the spectrum.

Format

Click on the Format left hand "Style" drop down box to set the number of display characters for the peak position value.



Click on the Format right hand "Style" drop down box to set the number of decimal places to be displayed for the peak position value.



Use data style

When "Use data style" is ticked the display color of the peak label text and arrow marker will be set to match the spectrum color. If "Use data style" is unticked the color will be set according to the selection made in the respective "Style" drop down box.

Shape multicolor

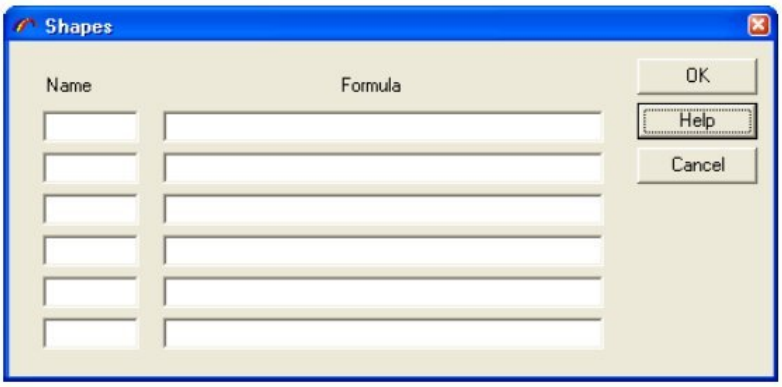
If "Shape multicolor" is ticked the individual peak shape display colour will be automatically selected from a default palette; in this case, when multiple shapes are displayed on a single spectrum each shape will be a different color. If "Shape multicolor" is unticked the colour will be set according to the selection made in the "Style" drop down box; in this case, when multiple shapes are displayed on a single spectrum each shape will be the same colour.

Attach arrow

If "Attach arrow" is ticked the peak arrow marker will be positioned immediately above the peak. If "Attach arrow" is unticked the peak arrow marker will be positioned at the top of the spectrum window.

4.6.7.5. Peak Functions Dialog Window

The Peak Functions dialog window allows custom peak fitting formulae to be defined, so that shapes other than the default Gaussian, Lorentzian and mixed Gaussian-Lorentzian can be used. For example, with the Peak Functions dialog window peak shapes such as Voigt or asymmetric Gaussian can be used.



To define a custom peak shape formula type the shape name in a "Name" text box, and input the formula in the "Formula" text box. The name will be displayed in the main "Functions" drop down box in the main Peaks dialog window.

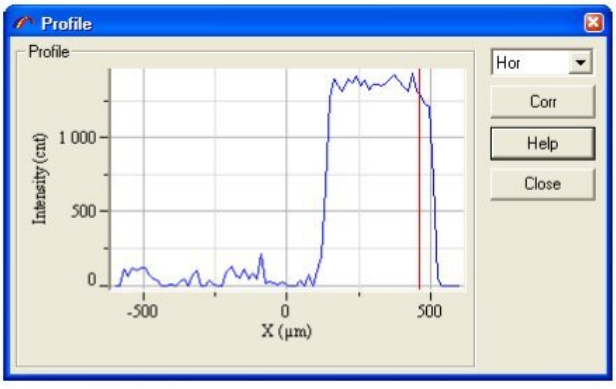
Any parameter can be used, but it must also be defined as a parameter in the Peak Variables dialog window (see section 4.6.7.3, page 140). Note that certain parameters are predefined:

- **p** – peak position, in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).
- **a** – peak amplitude, in units as displayed on the spectrum's Y axis, typically counts (cnt), or counts per second (cnt/s).
- **w** – peak full width at half maximum height (FWHM) in units as displayed on the spectrum's X axis, typically Raman shift (cm^{-1}) or nanometers (nm).

4.6.8. Profile

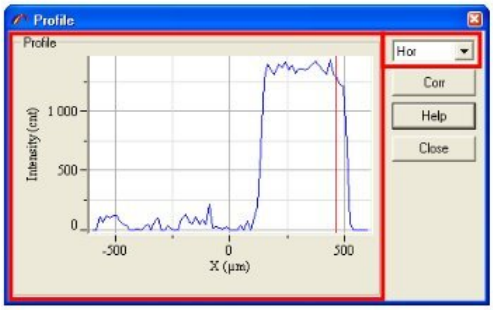
Opens the Profile dialog window.

The Profile dialog window displays an intensity profile across an image (such as optical image, or two dimensional Raman mapped image).



Profile

The "Profile" window displays the image intensity profile at the cursor position. The scales for the X and Y axes are taken directly from the image itself. The profile is created in either a horizontal (X axis) or vertical (Y axis) direction in the image, depending on the selection made in the drop down box:



- Hor – horizontal (X axis)
- Ver – vertical (Y axis)

Corr

Click on **[Corr]** to modify the image based on manipulation made to the profile. For example, if the profile displayed in the Profile dialog window is smoothed, clicking on **[Corr]** will apply the same smoothing function to the entire image in the horizontal or vertical dimensions (according to the selection of "Hor" or "Ver" in the drop down box).

4.6.8.1. Displaying an Intensity Profile from an Image

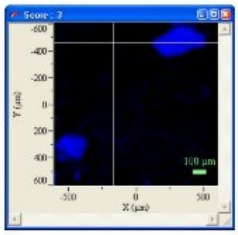
To display an intensity profile from an image (such as optical image, or two dimensional Raman mapped image) open the image file.

In the case of a two dimensional Raman mapped image click on the image window (either "Map" or "Score"). Select the component from which the profile is to be created, using the tags in the right hand Data bar (see section 6.3, page 198).

Ensure the cursor mode in the image window is set to "Cross" (right click and select "Cursor", and then choose "Cross" from the Style drop down box).

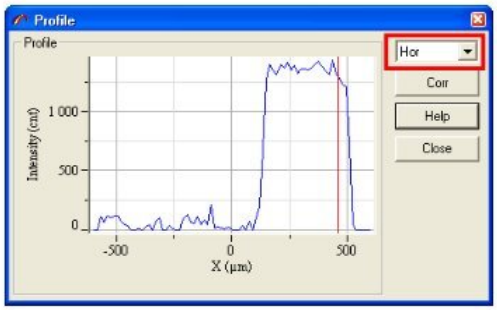
Open the Profile dialog window by clicking on the Profile icon in the Icon bar.

Position the cursor at the point of the image from where the profile is to be created.



Select “Hor” or “Ver” from the drop down box in the Profile dialog window to create an intensity profile in the horizontal (X axis) direction or vertical (Y axis) direction respectively.

The profile is displayed in the Profile dialog window. Standard data processing functions (such as smoothing, peak fitting or baselining) and copy/paste functions can be used with this profile.

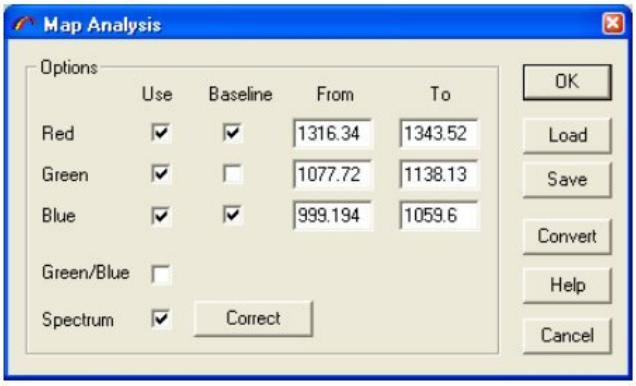


Click **[Close]** to close the Profile dialog window.

4.6.9. Map Analysis

Opens the Map Analysis dialog window.

The Map Analysis dialog window displays the positions and settings for the “Red”, “Green” and “Blue” cursors which are used to create intensity profiles and images from multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes). The positions and settings can also be manually configured in this dialog window.



Use

If the “Use” box is ticked for a set of cursors (“Red”, “Green” or “Blue”) then a profile or image will be generated displaying the average intensity between the two cursors. The cursor positions are set by clicking the “Red”, “Green” or “Blue” cursor icons in the left hand Graphical Manipulation bar (see section 5.2, page 165) and dragging the cursors to the desired positions on either the “SpIm” or “Point” windows, or by typing in desired values into the “From” and “To” boxes in the Map Analysis dialog window.

Note that if “Use” is unticked for all three cursors and “Green/Blue” is also unticked, the “Map” window will not be displayed. To redisplay the “Map” window make sure that at least one of the “Use” boxes are ticked, or that the “Green/Blue” box is ticked.

Baseline
If the "Baseline" box is ticked for a set of cursors ("Red", "Green" or "Blue") then the cursor region is first baselined before calculation of the average intensity between the cursors. This mode is useful to ensure that the image created truly reflects peak intensity and not general background intensity (perhaps from fluorescence or photoluminescence).

From and ***To***
Displays the beginning ("From") and end ("To") spectral positions for a set of cursors ("Red", "Green" or "Blue"). These can be manually adjusted by typing in desired values and clicking **[OK]**.

Green/Blue
If the "Green/Blue" box is ticked an additional profile/image is displayed, showing the ratio of average intensities of the "Green" and "Blue" cursors (i.e., [$Intensity_{GREEN}$] / [$Intensity_{BLUE}$]). This display is useful to visualize a change in peak ratios within a multidimensional spectral array.

To remove the "Green/Blue" intensity profile/image untick the box.

Spectrum
If the "Spectrum" box is ticked the "Point" window is displayed, showing the spectrum at the current cursor position.

Note that if the "Spectrum" box is unticked, the "Point" window will not be displayed. To redisplay the "Point" window tick the "Spectrum" box.

In the event that the "Point" window is accidentally deleted within the main LabSpec 5 graphical user interface (GUI), use the following procedure to re-display the window:

- open the Map Analysis dialog window
- untick the "Spectrum" box
- click **[OK]**
- re-open the Map Analysis dialog window
- tick the "Spectrum" box
- click **[OK]**
- the Point window is now displayed in the main LabSpec 5 graphical user interface (GUI)

Correct
Click on **[Correct]** to update the multidimensional spectral array with the "Point" spectrum after it has been processed/modified in some way (for example, smoothing or baselining).

The "Point" window only displays data held within the multidimensional spectral array, so if the spectrum in the "Point" window is modified it is necessary to update the actual data in the array by clicking on **[Correct]**. If this is not done, when the cursor on the profile/image is moved the modifications will be lost – the next time the spectrum is displayed in the "Point" window it will return to its original form.

Load

Click on **[Load]** to load a cursor parameters file (in .ngp format) so that a previously saved configuration of the Map Analysis dialog window can be recalled and applied.

Save

Click on **[Save]** to save a cursor parameters file (in .ngp format) so that the current configuration of the Map Analysis dialog window can be saved, and recalled at any time.

Convert

Click on **[Convert]** to convert an existing image file into the LabSpec 5 multidimensional spectral array format, including "SpIm", "Point" and "Map" windows.

4.6.10. Create Spectral Profile

Opens the Spectral Profile dialog window.

The Spectral Profile dialog window allows a 1D multidimensional spectral array to be created from individual spectra, and for spectra to be added to, deleted from, and inserted into an existing 1D multidimensional spectral array. This function is useful to create profiles from spectra where there is sequential change in some experimental parameter (e.g., reaction temperature, or concentration).

Name

Type the desired name for a new profile in the "Name" text box before clicking on **[New]** to create the profile.

New

Click on **[New]** to create a new spectral profile, using the active spectrum as the first spectrum in the profile.

If the "Multi" function is active (see section 3.4.2, page 23) then all open spectra will be added to the spectral profile when it is created. In this case, the active spectrum will be added first, followed by all other open spectra in the order they appear within the right hand Data bar.

See section 4.6.10.1, page 151, for further information.

Add

Click on **[Add]** to add the active spectrum to the end of the spectral profile.

See section 4.6.10.2, page 152, for further information.

Insert

Click on **[Insert]** to add the active spectrum to the spectral profile at the position shown by the cursor in the "Map" window.

See section 4.6.10.3, page 152, for further information.

Delete

Click on **[Delete]** to delete a spectrum from the spectral profile. The spectrum at the position shown by the cursor in the "Map" window will be deleted.

See section 4.6.10.4, page 153, for further information.

XYZ

Click on **[XYZ]** to rotate an XYZ multidimensional spectral array so that the data is formatted in the default Z.Y.X format (e.g., a Z-stack of YX maps). See section 4.5.5.3, page 99, for information about controlling the acquisition order for multidimensional spectral arrays.

4.6.10.1. Creating a New Spectral Profile

Using File > Open menu, or the Open icon (/) open the individual spectra which are to be used to create the spectral profile.

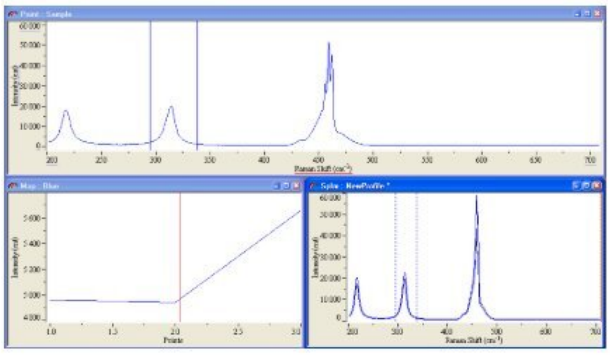
Open the Spectral Profile dialog window by clicking on the Create Spectral Profile icon.

Type into the “Name” text box a name for the new profile.

Select the first spectrum to be added to position 1 in the new profile, and click **[New]** to create the profile. The new profile will be created in two ways, depending whether the “Multi” function is active (see section 3.4.2, page 23):

- “Multi” active: the active spectrum will be added to position 1 in the profile, followed by all other open spectra, in the order they appear within the right hand Data bar.
- “Multi” inactive: the active spectrum will be added to position 1 in the profile. No other spectra will be added to the profile. Additional spectra can be added to the profile using the **[Add]** button (see section 4.6.10.2, page 152) or **[Insert]** button (see section 4.6.10.3, page 152).

When the profile is created, the standard “SpIm”, “Point” and “Map” windows associated with a multidimensional spectral array will be displayed. The profile can now be analysed in the normal way, using cursors and modelling.



The profile will be created with each spectrum assigned to an integer value on the X axis, and the X axis label is set to "Points". To modify the scaling and axis label use the Data Range dialog window, accessed through the Data > Data Range menu, the Data Range icon (/), or by using the keyboard short cut <CTRL>+D. See section 3.3.2, page 20, for more information about the Data Range dialog window.

Save the spectral profile (in .ngc format) by activating the "SpIm" window and using the File > Save As menu, or the Save icon (/).

4.6.10.2. Adding Spectra to a Spectral Profile

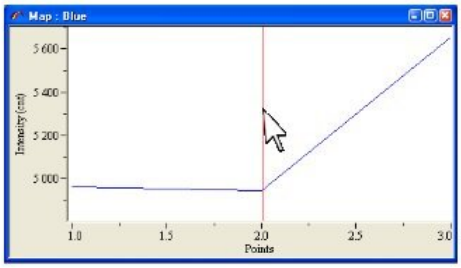
To add a spectrum into an existing Spectral Profile, use File > Open menu, or the Open icon (/), or the keyboard short cut <CTRL>+O to open the spectrum which is to be added.

Select the spectrum, and click on **[Add]** to automatically add the spectrum at the end of the spectral profile. For example, if the profile already has spectra at positions 1, 2 and 3, a new spectrum added to the profile will be at position 4.

4.6.10.3. Inserting Spectra into a Spectral Profile

To insert a spectrum into an existing Spectral Profile, use File > Open menu, or the Open on (/), or the keyboard short cut <CTRL>+O to open the spectrum which is to be inserted.

In the profile "Map" window, activate the cursor and select the position at which the new spectrum is to be inserted.



Select the spectrum which is to be inserted, and click on **[Insert]** to insert it at the position indicated by the cursor.

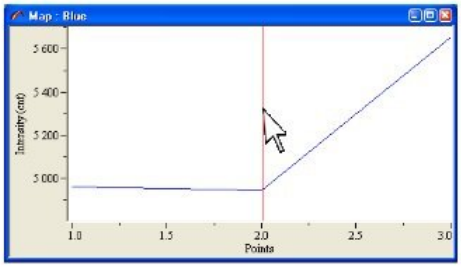
In a default spectral profile, the other spectra originally at and above this position will be shifted

upwards in the profile. For example, if spectra A, B and C are currently at positions 1, 2 and 3 in a profile of form 1A, 2B, 3C, when spectrum D is inserted at position 2, the new profile will take the form 1A, 2D, 3B, 4C.

In a spectral profile where the scale has been manually modified using the Data Range dialog window (see section 3.3.2, page 20), the first and last positions of the profile will remain fixed. The positions of spectra within the profile will be adjusted when a spectrum is inserted. For example, if spectra A, B and C are currently at positions 1, 2 and 3 in a manually scaled profile of form 1A, 2B, 3C, when spectrum D is inserted at position 2, the new profile will take the form 1A, 1.66D, 2.33B, 3C. If necessary the profile can be rescaled using the Data Range dialog window after the spectrum has been inserted.

4.6.10.4. Deleting Spectra from a Spectral Profile

In the profile "Map" window, activate the cursor and select the position from which a spectrum is to be deleted.



Click on **[Delete]** to delete the spectrum from the position indicated by the cursor position.

In a default spectral profile, the other spectra above this position will be shifted downwards in the profile. For example, if spectra A, B, C and D are currently at positions 1, 2, 3 and 4 in a profile of form 1A, 2B, 3C, 4D, when spectrum B is deleted, the new profile will take the form 1A, 2C, 3D.

In a spectral profile where the scale has been manually modified using the Data Range dialog window (see section 3.3.2, page 20), the first and last positions of the profile will remain fixed. The positions of spectra within the profile will be adjusted when a spectrum is deleted. For example, if spectra A, B, C and D are currently at positions 1, 2, 3 and 4 in a manually scaled profile of form 1A, 2B, 3C, 4D when spectrum B is

deleted, the new profile will take the form 1A, 2.5C, 4D. If necessary the profile can be rescaled using the Data Range dialog window after the spectrum has been deleted.

4.6.11. Modelling

Opens the Model dialog window.

The Model dialog window is used to set up and perform a direct classical least squares (DCLS) modelling procedure on multidimensional spectral arrays (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes) using a set of reference component spectra. This procedure is used to identify the distribution of the reference component spectra within the spectral array to create a profile/image based on the component distribution. The component spectra can be either manually selected (from previously saved spectra, or from within the spectral array) or automatically created by LabSpec 5 using a clustering algorithm.

4.6.11.1. The DCLS Modelling Procedure

At each position within the multidimensional spectral array the DCLS modelling procedure finds a linear combination of the reference component spectra which best fits the raw data. The resulting profile/image is created by showing the contribution ('score') of each component ('loading') as a profile/image.

For example, if there are three loadings (A, B, and C) with scores (x, y and z) the sum, S, of the linear combination is represented by:

$S = [x * A] + [y * B] + [z * C]$

A, B, C are the loadings, e.g., reference spectra of pure components.
x, y, z are the scores, e.g., the 'amount' of each loading necessary so that S matches the raw data.

Normalized Modelling
When the modelling procedure is normalized (i.e., the "Normalize" box is ticked prior to performing the modelling procedure), the reference component spectra (loadings) are normalized before the modelling procedure takes place.

The scores are normalized so that the combination of all scores adds to 100%. If normalization is turned off after normalized modelling has been performed, the scores are shown as their true values.

Unnormalized Modelling
When the modelling procedure is unnormalized (i.e., the "Normalize" box is unticked prior to performing the modelling procedure), the reference component spectra (loadings) are used with their raw intensities during the modelling procedure. Unnormalized modelling must be used if quantitative analysis is required, since the actual intensity of each reference component spectrum relates directly to the its concentration.

The scores are shown as their true values. If normalization is turned on after unnormalized modelling has been performed, the scores are displayed as normalized values so that the combination of all scores adds to 100%.

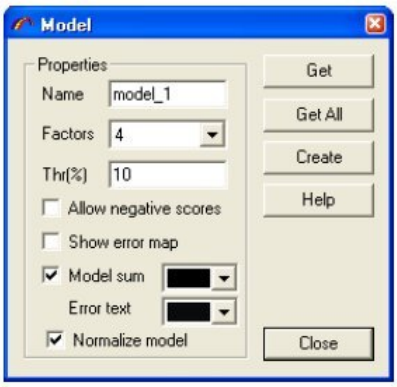
4.6.11.2. The "Create" Clustering Procedure

The "Create" clustering procedure automatically identifies a number of reference component spectra (loadings) using a factor analysis algorithm. This algorithm searches the entire multidimensional spectral array and locates a number of clusters of similar spectra, where the average of each cluster is as different from the other cluster averages as possible. The number of clusters is specified in the "Factors" drop down box.

Once the average spectra have been identified in this way they are used as loadings in the DCLS modelling procedure, as described above (section 4.6.11.1, page 154).

4.6.11.3. The Model Dialog Window

The Model dialog window allows reference component spectra to be manually added to the modelling procedure, in addition to automatic generation of component spectra with subsequent modelling using the "Create" clustering procedure. The display mode of the modelling result can also be configured through the dialog window.



Name
Type in a name in the "Name" text box for each reference component spectrum to be used in the modelling procedure, prior to clicking on **[Get]** to start the modelling. See section 4.6.11.4, page 157, for more information about how to use the modelling procedure.

Factors
Select the number of factors to be automatically created using the "Create" clustering procedure. See section 4.6.11.5, page 161, for more information about how to use the automatic "Create" clustering procedure.

Thr(%)
Type in a value (in percent, %) in the "Thr(%)" text box to set the intensity threshold for the automatic "Create" clustering procedure. The threshold ensures that low intensity spectra will be excluded from the "Create" clustering procedure – spectra with a maximum signal level less than the threshold value (in percent, %) of the highest signal intensity within the entire spectra array will be ignored.

Allow Negative Scores
If "Allow negative scores" is ticked the scores for the loadings can take both positive and negative values. If it is unticked the scores for the loadings can only take positive values. See section 4.6.11.1, page 154, for an explanation of the modelling procedure, and what the 'scores' and 'loadings' are.

Show Error Map
If "Show error map" is ticked an additional score profile/image will be displayed based on the error between the sum of the linear combination and the raw data. Regions of high error (bright intensity) indicate a bad fit, and could be caused by a missing reference component spectrum.

To remove the error profile/image untick the "Show error map" box.

Model Sum
If "Model sum" is ticked the sum of the linear combination will be displayed in the "Point" window, in addition to the score/loading information. Select the colour for the sum spectrum from the drop down box.

Error Text
Select the text colour from the drop down box for the error value displayed in the "Point" window.

Normalize Model
The normalization mode for the modelling procedure can be controlled using the "Normalize model" tick box. See section 4.6.11.1, page 154, for more information about the modelling procedure and normalization.

Get
Click on **[Get]** to load the currently active spectrum as a reference component spectrum and start the modelling procedure. See section 4.6.11.4, page 157, for more information about how to use the modelling procedure.

Get All
Click on **[Get All]** to load in multiple model spectra saved from a previous modelling procedure, and start a new modelling procedure using them. This function is useful to apply a modelling procedure to a spectral array using identical reference component spectra used for previous spectral array modelling.

See section 4.6.11.4.1, page 160, for more information about using the **[Get All]** button to start a modelling procedure with previously saved reference component spectra.

Create

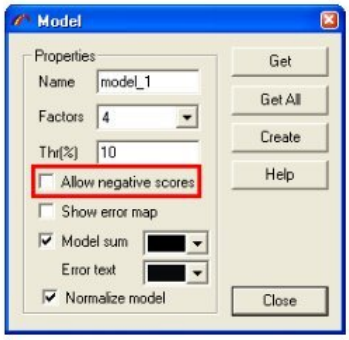
Click on **[Create]** to start the automatic "Create" clustering procedure and subsequent modelling. See 4.6.11.5, page 161, for more information about how to use the automatic "Create" clustering procedure

4.6.11.4. How to Model a Multidimensional Spectral Array

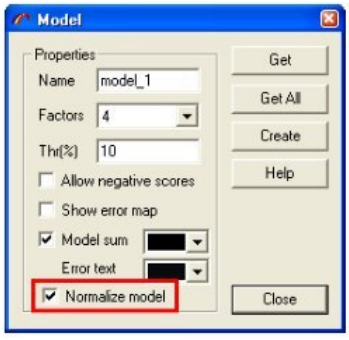
The DCLS modelling procedure can be used with any multidimensional spectral array (including time profiles, Z (depth) profiles, temperature profiles, XY maps, XZ and YZ slices, and XYZ datacubes). The procedure described here assumes that a spectral array file is currently open, with the "SpIm", "Point" and "Map" windows visible.

Open the Model dialog window by clicking on the Modelling icon.

Select whether loading scores can take both positive and negative values, or just positive values using the "Allow negative scores" tick box. If "Allow negative scores" is ticked the scores for the loadings can take both positive and negative values. If it is unticked the scores for the loadings can only take positive values. See section 4.6.11.1, page 154, for an explanation of the modelling procedure, and what the 'scores' and 'loadings' are. In general "Allow negative scores" should be unticked, and it is recommended that it is ticked for specialized analysis only.



Select the normalization mode of the modelling procedure, by either ticking or unticking the "Normalize model" box. For general analysis of the spectral array, and characterisation of component distribution normalized modelling will be suitable. If quantitative analysis of component distribution within the spectral array is required, unnormalized modelling must be used. See section 4.6.11.1, page 154, for more information about the normalization modes.



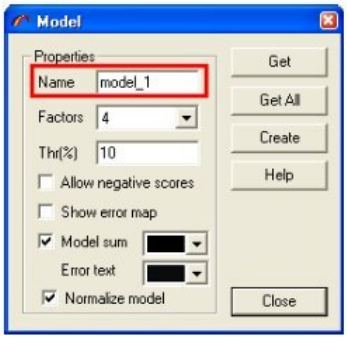
Select a spectrum which is to be used as a reference component spectrum in the modelling procedure. The spectrum can be selected in two ways:

- Locate a 'pure' reference component spectrum from within the spectral array using the cursor in the "Map" window.

Click on the "Point" window to select the spectrum.

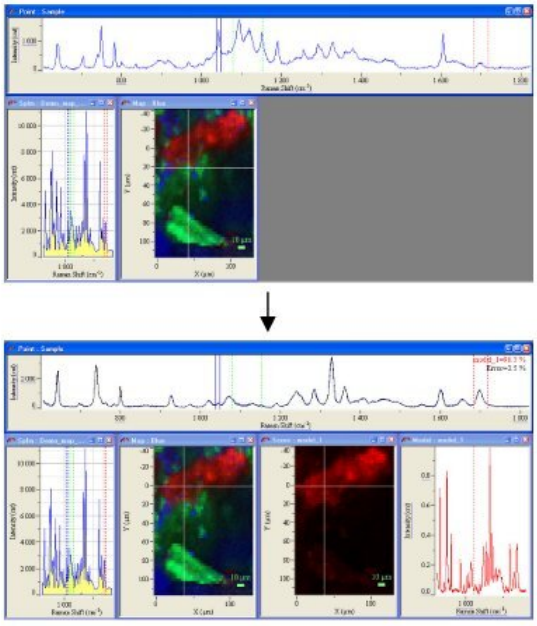
- Open an existing spectrum file (saved in any standard LabSpec spectrum format) using File > Open, or the Open icon (/), or the keyboard short cut <CTRL>+O. Click on the spectrum window and select the spectrum. If multiple spectra are opened, ensure the correct spectrum is selected.

In the Model dialog window type in a name for the reference component spectrum in the "Name" text box. This step is not essential, but good naming of reference component spectra can assist analysis and understanding of the results obtained at the end of the modelling procedure. If a name is not needed, then ensure the "Name" text box is blank – delete any entry which is already present.



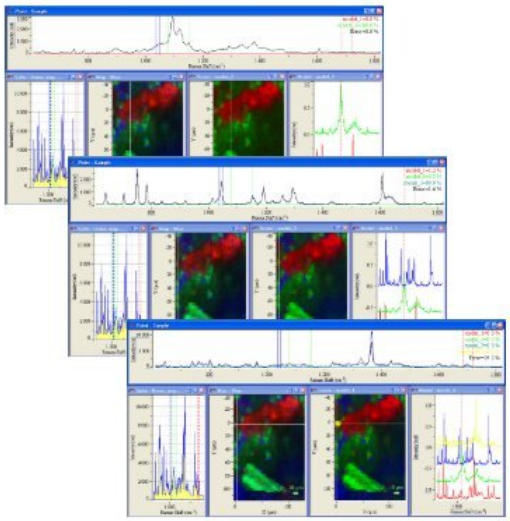
Click on **[Get]** in the Model dialog window. The selected spectrum will be loaded, and the modelling procedure will be started. Two new windows will be created in addition to the standard "SpIm", "Point" and "Map" windows:

- "Scores" window – displays the profile/image based on the loading scores calculated by the modelling procedure.
- "Model" window – displays the reference component spectra used by the modelling procedure.



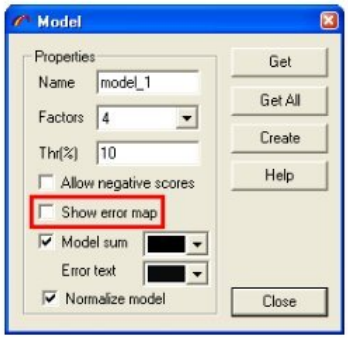
Note that the cursors displayed in the "Map" and "Score" windows are linked, and both will move when one is manipulated with the mouse.

Repeat the process to add more reference component spectra to the modelling procedure, by selecting (one at a time) additional spectra and clicking on **[Get]** for each spectrum. The "Score" and "Model" window will update each time.



Continue until all necessary reference component spectra have been included in the model for the spectral array.

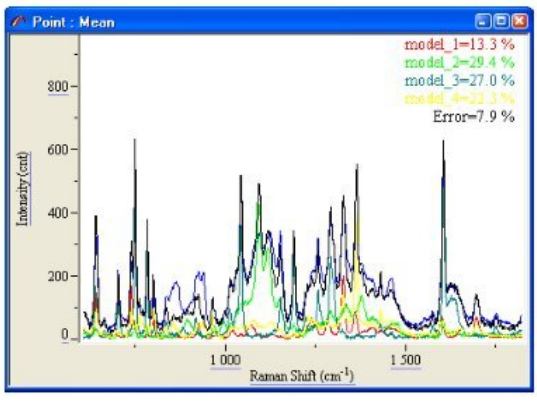
The error profile/map can be used to check that there are no regions of high error, which often indicates that a further reference component spectrum is necessary. The error profile/map can be activated by ticking the "Show error map" box. It will be displayed as another colored image in the "Score" window.



Once the modelling procedure has been completed the "Point" window displays the following spectra:

- Raw data: ———
- Sum: * ———
- Components: ⁺ — — —

In addition, in the top right hand corner of the window a summary of the modelling result is displayed. This shows the contribution of each reference component spectrum, and the error.‡ The contribution is displayed as actual score (if "Normalize model" is unticked) or as relative percent (if "Normalize model" is ticked).



[*] Sum spectrum will only be displayed if "Model sum" is ticked, and its color will be according to the color drop down box.

[†] Components will be sequentially colored using a default palette. The first three components are displayed as red, green, and blue.

[‡] The display color of the error text will be according to the "Error text" color drop down box.

The modelling results can be saved with the spectral array data by activating the "SpIm" window and saving the file in LabSpec 5 .ngc format. The save dialog window is accessed using File > Save As..., clicking on the Save icon (/), or using the keyboard shortcut <CTRL>+S. When the spectral array file is next opened the "Score" and "Model" windows will also open.

4.6.11.4.1. Using a Previously Saved Set of Reference Component Spectra

The *How to Model a Multidimensional Spectral Array* procedure outlined above is based on each reference component spectrum being selected and modelled individually, on a one by one basis. It is also possible to recall a previously used set of reference component spectra, and load all spectra simultaneously. This is useful if you wish to model a number of spectral arrays in exactly the same way, using exactly the same reference component spectra.

Saving the Reference Component Spectra

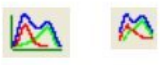
To save a set of reference component spectra associated with a multidimensional spectral array model, activate the "Model" window, and save the reference component spectra as a single file (in .ngs or .tsf formats) using File > Save All.

Opening and Loading the Reference Component Spectra

The procedure described here assumes that a spectral array file is currently open, with the "SpIm", "Point" and "Map" windows visible.

Open the single file (in .ngs or .tsf format) containing the reference component spectra using File > Open, the Open icon (/), or the keyboard short cut <CTRL>+O. The spectra will be opened within a "Model" window.

Open the Model dialog window by clicking on the Modelling icon.



Activate the "Model" window, and click on **[Get All]** in the Model dialog window.

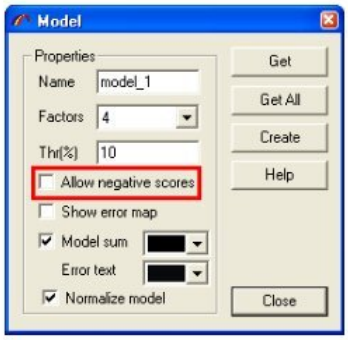
The modelling procedure will be launched using all of the reference component spectra. The "Score" and "Model" windows will be created once the modelling procedure is completed.

4.6.11.5. How to Automatically Create a Model for a Multidimensional Spectral Array

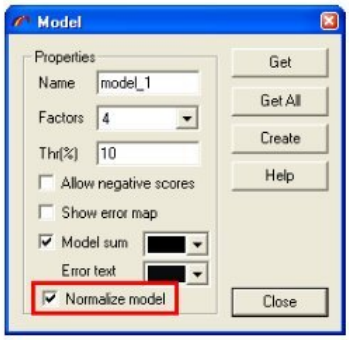
The procedure described here assumes that a spectral array file is currently open, with the "SpIm", "Point" and "Map" windows visible.

Open the Model dialog window by clicking on the Modelling icon.

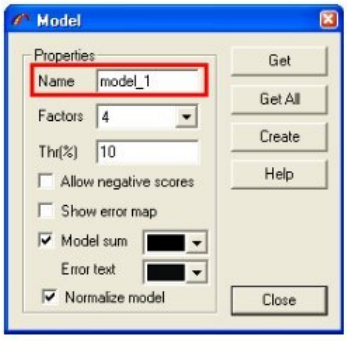
Select whether loading scores can take both positive and negative values, or just positive values using the "Allow negative scores" tick box. If "Allow negative scores" is ticked the scores for the loadings can take both positive and negative values. If it is unticked the scores for the loadings can only take positive values. See section 4.6.11.1, page 154, for an explanation of the modelling procedure, and what the 'scores' and 'loadings' are. In general "Allow negative scores" should be unticked, and it is recommended that it is only ticked for specialized analysis only.



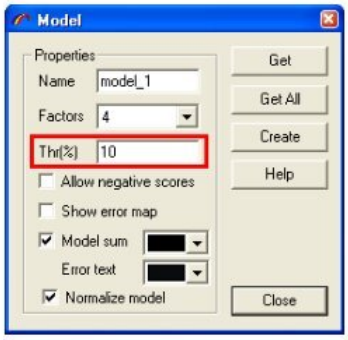
Select the normalization mode of the modelling procedure, by either ticking or unticking the "Normalize model" box. For general analysis of the spectral array, and characterisation of component distribution normalized modelling will be suitable. If quantitative analysis of component distribution within the spectral array is required, unnormalized modelling must be used. See section 4.6.11.1, page 154, for more information about the normalization modes.



Type in a generic name for the component spectra in the "Name" text box. The created component spectra will be labelled sequentially in the form name_1, name_2 etc. This step is not essential, but good naming of reference component spectra can assist analysis and understanding of the results obtained at the end of the modelling procedure. If a name is not needed, then ensure the "Name" text box is blank – delete any entry which is already present.

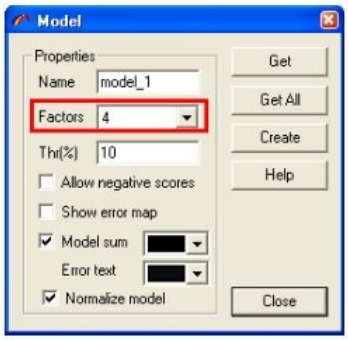


Type in a value (in percent, %) in the "Thr(%)" text box to set the intensity threshold for the "Create" clustering procedure. The threshold ensures that low intensity spectra will be excluded from the "Create" clustering procedure – spectra with a maximum signal level less than the threshold value (in percent, %) of the highest signal intensity within the entire spectra array will be ignored. For general analysis 10% is suitable, but the value should be adjusted as required.



Select from the "Factors" drop down box the number of component spectra which are to be created using the clustering algorithm.

Note that the number of component spectra should be chemically meaningful. If in doubt, start with a small number, and increase it if the modelling results do not look correct.



Click on **[Create]** to start the "Create" clustering procedure, and subsequently launch the modelling procedure using the created component spectra. The "Score" and "Model" windows showing the results will be created once the modelling procedure has been completed.

The modelling results can be saved with the spectral array data by activating the "SpIm" window and saving the file in LabSpec 5 .ngc format. The save dialog window is accessed using File > Save As..., clicking on the Save icon (/), or using the keyboard shortcut <CTRL>+S. When the spectral array file is next opened the "Score" and "Model" windows will also open.

4.7. Stop Active Function Icon

4.7.1. Stop Active Function

Stop the current software function (including data acquisition and data processing).

5. Graphical Manipulation Toolbar

The Graphical Manipulation toolbar located on the left hand side of the LabSpec 5 graphical user interface (GUI) provides access to a range of spectrum manipulation and analysis functions.

This is an active toolbar, and its appearance and content will update according to the currently selected window. For example, the options appearing in this toolbar when a spectrum is active will differ from those appearing when a video image is active.

Note that when clicked an icon will be locked down. Only one icon can be active and locked down at a time.

At the end of each icon's description, a list of windows where the icon is available in the Graphical Manipulation toolbar is given. The possible windows are as follows:

Spectrum
The spectrum display window for individual spectra acquired using the real time display (RTD) acquisition (/) and spectrum acquisition (/) modes.

Video
The video display window for optical images acquired with the integrated microscope camera(s).

SpIm
The overlay of all spectra within a multidimensional spectral array.

Point
The spectrum at the current cursor position within a multidimensional spectral array.

Map
The cursor intensity profile/image display created from a multidimensional spectral array.

Score
The score profile/image created by DCLS modelling of a multidimensional spectral array

Model
The reference component spectra used for DCLS modelling of a multidimensional spectral array.

5.1. Pointer

Activates the cursor (pointer) so that individual X axis and/or Y axis values can be read from the window. The cursor values will be displayed in the Status bar (see section 7.5, page 201).

The cursor can be configured using the Cursor dialog window, by right clicking and selecting "Cursor" - see section 8.11, page 218. A number of cursor modes are available:

Spectrum, SpIm, Point, Model windows

- Line – single vertical line cursor, displaying the X axis position (S) of the cursor and the intensity (I) of the spectrum at the cursor position.
- Cross – cross hair cursor, displaying the X axis position (S) and Y axis position (I) of the cursor.
- Level – cross hair cursor which tracks the intensity of the spectrum, displaying the X axis position (S) and Y axis position (I) of the cursor. In this case, the Y axis position is equivalent to the spectrum intensity at the cursor position.
- Double – two vertical cursors, displaying the X axis position (S) of each cursor and the width between the two cursors (W).
- Peak – three linked vertical cursors, the central one locking to the maximum intensity pixel in a peak, and the outer two locking to the pixels closest to the full width at half maximum height (FWHM) of the peak. The Peak cursor displays the X axis position (S), intensity (I) and approximate full width at half maximum height (W) of the peak at the cursor position.

Video, Map, Score windows

- Cross – cross hair cursor, displaying the X axis position (X) and Y axis position (Y) of the cursor, and pixel intensity (I) at the cursor position. For the Map and Score windows, the spectrum associated with the cursor position will be displayed in the Point window.
- Rect – rectangular cursor (resizeable by left clicking and dragging the drag points), displaying the X axis position (X) and Y axis position (Y) of the bottom, right hand corner of the rectangular cursor. For the Map and Score windows, the average spectrum from within the rectangle is displayed in the Point window.

Left click to position the cursor at any point on the spectrum/profile/image, or alternatively left click and drag to move the cursor to the desired position.

If one or both of the cursors are not visible on the spectrum/image, do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.


- Right click and select "Center cursor".

Available for: *Spectrum, Video, Model*

5.2. Map Analysis Cursors (SpImRed, SpImGreen, SpImBlue)

Activates the map analysis cursors, allowing profiles/images corresponding to the average intensity between the cursor pairs to be generated for multidimensional spectral arrays. Three map analysis cursor pairs are available: Red (SpImRed), Green (SpImGreen) and Blue (SpImBlue).

Left click to position the closest cursor of the pair at the click position, or alternatively left click and drag to move the closest cursor to the desired position.

Note that the map analysis cursors can be set to have a fixed width. To do this, right click and select "Red cursor", "Green cursor" or "Blue cursor" and tick "Fixed width". When the cursors have a fixed width, left click and drag to scroll the two cursors along the spectrum. In this case, it is not possible to individually position each cursor in the pair.

If one or both of the cursors are not visible on the spectrum/image, do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.


- Right click and select "Center cursor".

The map analysis cursors should be operated in "Double" mode – right click and select "Red cursor", "Green cursor" or "Blue cursor", and select "Double" from the "Style" drop down box.

The Map Analysis cursors should be used in conjunction with the Map Analysis dialog window- see section 4.6.9, page 147.

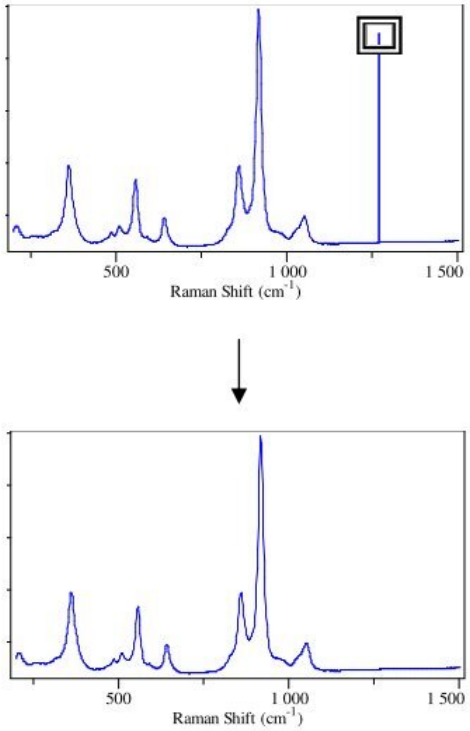
Available for: *SpIm, Point*

5.3. Remove Spike

Activates a spike removal tool, allowing random spikes (also known as cosmic rays) to be manually removed from a spectrum.

When this icon is active, the mouse cursor changes to the Remove Spike cursor.

Left click the Remove Spike cursor on a spike in the spectrum to remove it.



Note that if used on a true Raman peak the Remove Spike tool will modify the peak shape and intensity; it should only be used on spikes, and should be used with care.

Please see the following sections for other spike removal tools available in LabSpec 5:

Spike Filter	section 3.5.4.7, page 37
Despike	section 4.6.4.1.2, page 125
Denoise	section 3.5.4.15, page 46, and section 4.6.4.1.1, page 124

Available for: Spectrum, Point, Model

5.4. Correct Shape

Activates a pencil drawing tool, allowing manual modification of spectral features.

When this icon is active, the mouse cursor changes to the Correct Shape cursor.

Left click and drag the Correct Shape cursor to draw the new spectrum shape as desired.

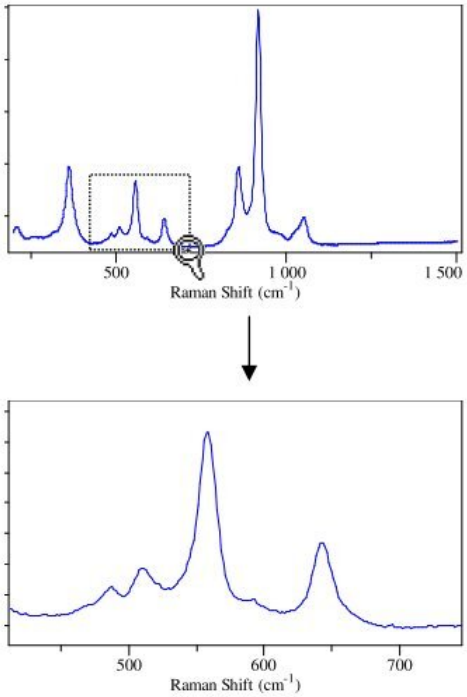
Available for: Spectrum, Point, Model

5.5. Zoom

Activates the Zoom cursor, allowing a small area of a spectrum/profile/image to be studied in detail. This icon can be activated with the <CTRL>+M keyboard shortcut.

When this icon is active, the mouse cursor changes to the Zoom cursor.

Left click and drag the Zoom cursor to select the area which will be displayed in the window.



To rescale the window do one of the following:

- Click on the "Scale Normalization" icon in the Icon bar– see 4.4.1, page 91.

- Use the <CTRL>+N keyboard short cut for "Scale Normalization".
- Right click and select "Rescale"

Available for: *Spectrum, Video, SpIm, Point, Map, Score, Model*

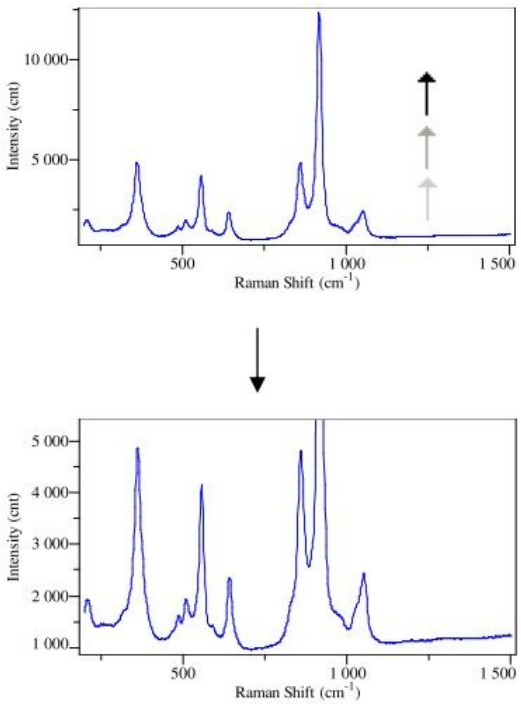
5.6. Intensity Shift

Activates the Intensity Shift cursor, allowing the upper display limit of the intensity axis (Y axis for spectra and profiles, Z axis for images) to be manually scaled.

When this icon is active, the mouse cursor changes to the Intensity Shift cursor.

Left click and drag the Intensity Shift cursor on the spectrum, profile or image window to adjust the scaling.

- Dragging upwards (as in the example shown right) will make the upper display limit of the intensity axis be reduced. This is equivalent to zooming in on the intensity axis; for images the affect will be to brighten the image.
- Dragging downwards will make the upper display limit of the intensity axis be increased. This is equivalent to zooming out on the intensity axis; for images the effect will be to darken the image.



Note that this function is purely a display function – it does not affect the actual data of the spectrum.

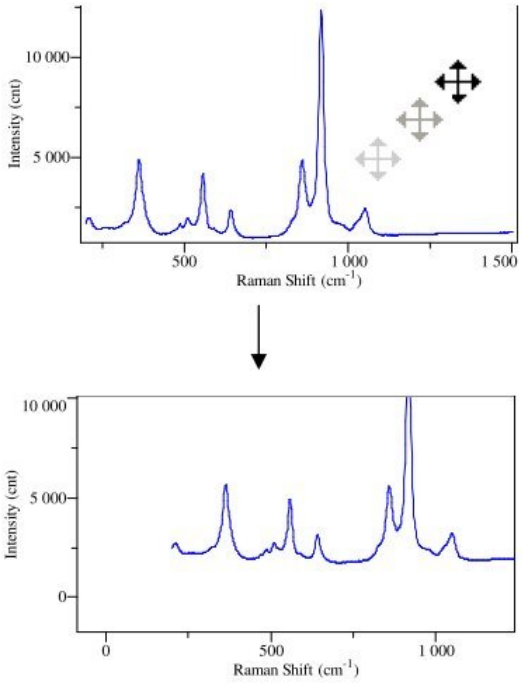
Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

5.7. Scale Shift

Activates the Scale Shift cursor, allowing the display scales of the X and Y axes to be shifted to higher or lower values.

When this icon is active, the mouse cursor changes to the Scale Shift cursor.

Left click and drag the Scale Shift cursor on the spectrum, profile or image window to shift scales of the X and Y axes.



To reset the X and Y axes scales do one of the following:

- Click on the "Scale Normalization" icon in the Icon bar– see section 4.4.1, page 91.

- Use the <CTRL>+N keyboard short cut for "Scale Normalization".
- Right click and select "Rescale"

Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

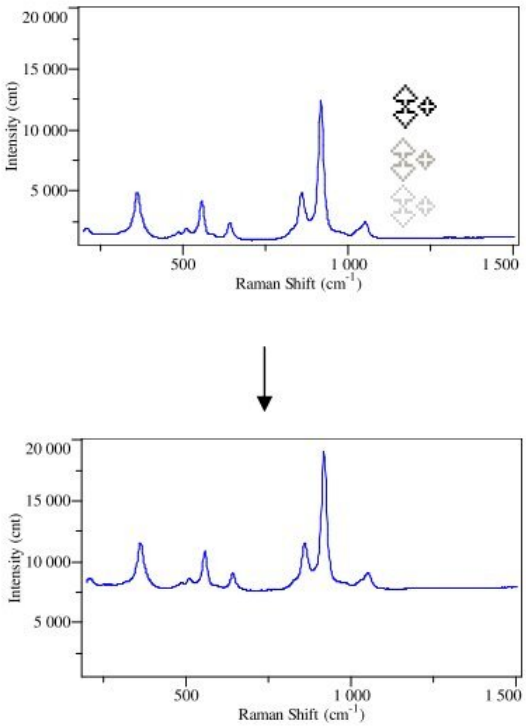
5.8. Add Constant

Activates the Add Constant cursor, allowing a constant intensity value to be added to or subtracted from all pixels in the active spectrum (i.e., to shift the entire spectrum up or down in the intensity (Y) axis).

When this icon is active, the mouse cursor changes to the Add Constant cursor.

Left click and drag the Add Constant cursor on the spectrum window to shift the spectrum up or down.

- Dragging upwards (as in the example shown right) will shift the spectrum upwards (to a higher intensity position), equivalent to adding a constant to the spectrum
- Dragging downwards will shift the spectrum downwards (to a lower intensity position), equivalent to subtracting a constant from the spectrum.



This function is related to the "Const+" function available in the Math dialog window - see section 4.6.6, page 130.

Available for: *Spectrum, Point, Model*

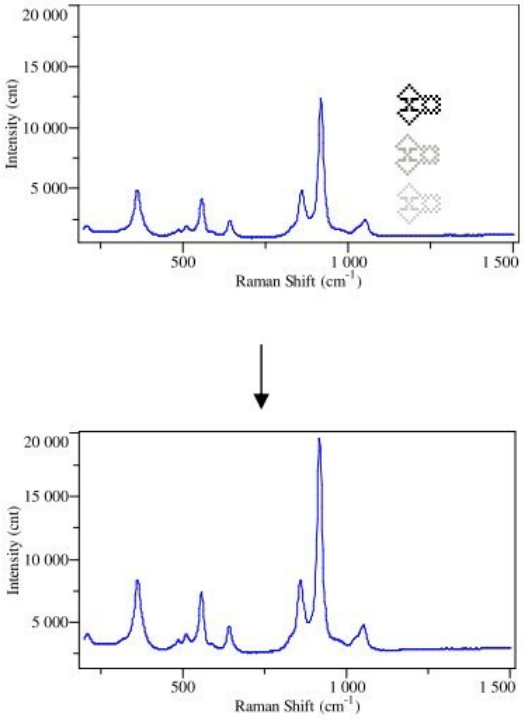
5.9. Multiply by Constant

Activates the Multiply by Constant cursor, allowing all pixels in the active spectrum to be multiplied or divided by a constant value to be added to or subtracted from all pixels in the active spectrum (i.e., to increase or decrease the entire spectrum intensity).

When this icon is active, the mouse cursor changes to the Multiply by Constant cursor.

Left click and drag the Multiply by Constant cursor on the spectrum window to shift the spectrum up or down.

- Dragging upwards (as in the example shown right) will increase the spectrum intensity, equivalent to multiplying the spectrum by a constant.
- Dragging downwards will decrease the spectrum intensity, equivalent to dividing the spectrum by a constant.



This function is related to the "Const*" function available in the Math dialog window - see section 4.6.6, page 130.

Available for: *Spectrum, Point, Model*

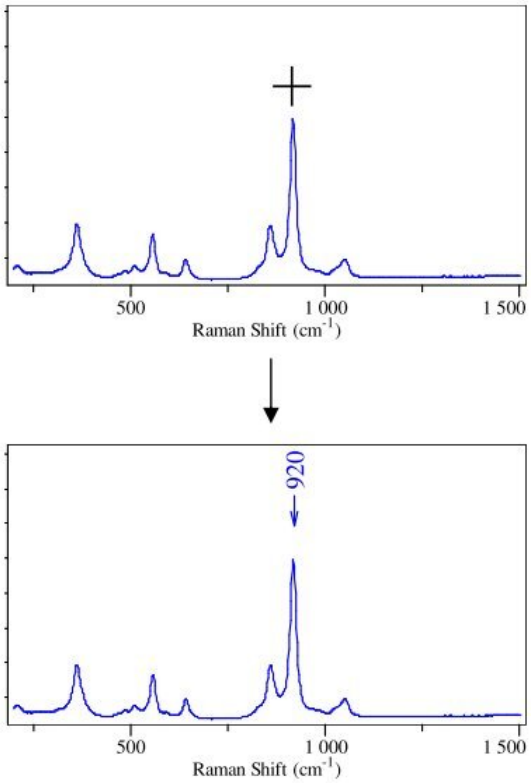
5.10. Add Peak

Activates the Add Peak cursor, allowing manual labelling of a peak position on the spectrum.

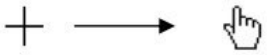
When this icon is active, the mouse cursor changes to the Add Peak cursor. 

Left click the Add Peak cursor at the desired spectrum position to label a peak. The peak label will be positioned in the X axis according to the click position; the Y (intensity) axis position will be automatically set according to the spectrum intensity at that position.

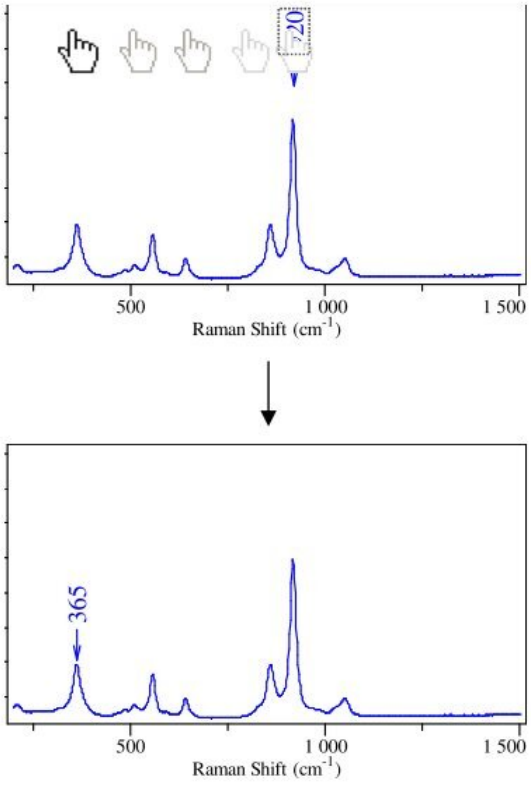
Note that the actual display of the peak label will depend on the settings in the Peak Options dialog window. See section 4.6.7.4, page 143.



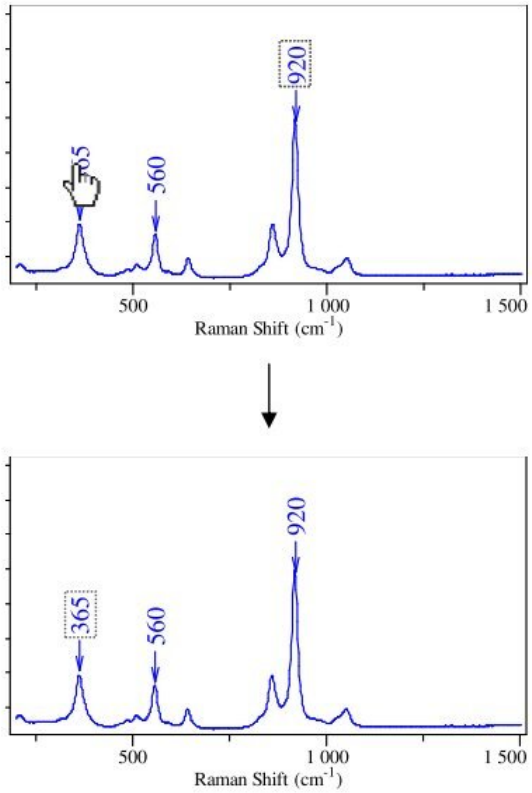
When the Add Peak cursor is active, and the mouse is hovered over an existing peak label, the cursor changes to a Move Peak cursor.



Left click and drag the Move Peak cursor to move the peak label to a different position on the spectrum.



When multiple peaks are labelled on a spectrum, the active peak label is enclosed in a dashed box. Use the Move Peak cursor to activate a different peak label, by left clicking on that peak label.



The Add Peak and Move Peak cursors should be used in conjunction with the Peak Searching and Fitting module - see section 4.6.7, page 134.

Available for: *Spectrum, SpIm, Point, Model*

5.11. Adjust Peak

Activates the Adjust Peak cursor, allowing peak fit parameters to be manually adjusted prior to fitting. The peak position, amplitude and full width at half maximum height (FWHM) can be adjusted using this cursor.

Typically this function requires that the peak shape is visible on the spectrum, so that the shape and position can be manually adjusted to approximately fit the raw data. The peak shape display can be set in the Peak Options dialog window – see section 4.6.7.4, page 143.

When this icon is active, the mouse cursor changes to the Adjust Peak cursor. Depending on the position of the mouse on the spectrum, this cursor has three possible forms:

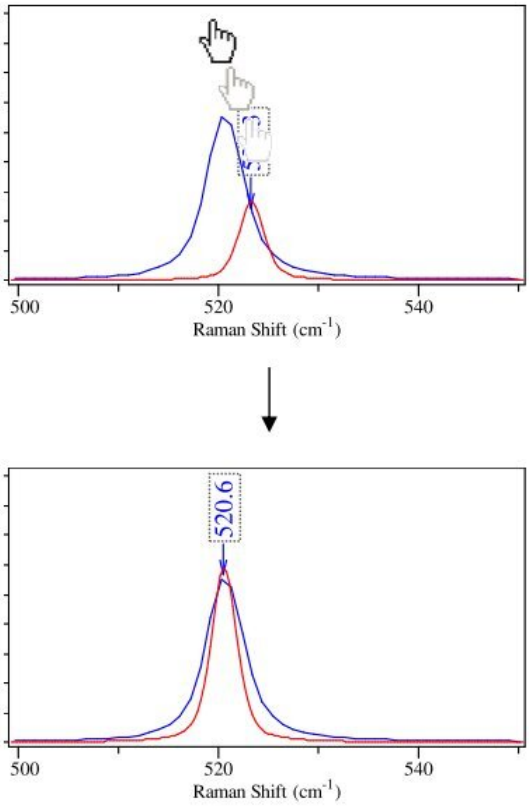
- When the mouse hovers over a peak label the Adjust Position cursor is displayed.

- When the mouse hovers to the left of a peak label the Adjust Width cursor is displayed.

- When the mouse hovers to the right of a peak label the Adjust Width cursor is displayed.

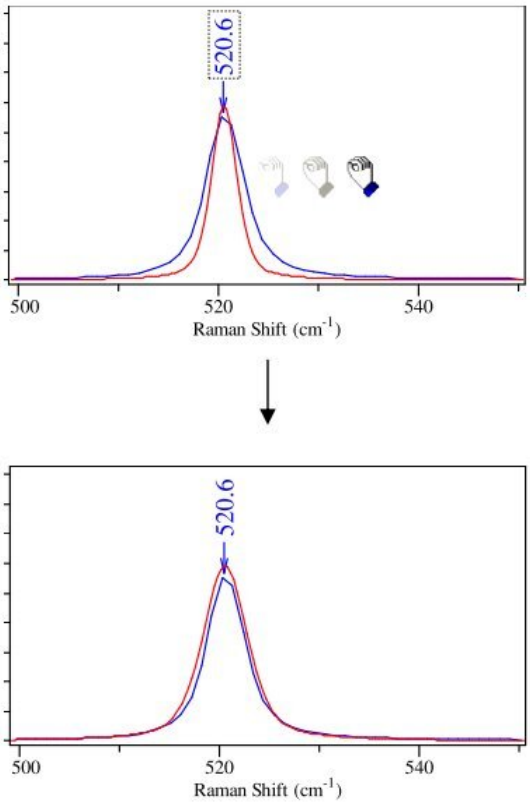
Adjustment to a peak shape and position is only possible for the active peak label/shape. In a spectrum with multiple peak labels/shapes, use the Add Peak / Move Peak cursor to activate the peak which is to be adjusted – see section 5.10, page 172.

Left click and drag on the peak label to adjust its position and amplitude.



Left click and drag on either side of the peak to adjust the peak full width at half maximum height (FWHM).

- Dragging away from the peak (as in the example shown right) will increase the full width at half maximum height (FWHM).
- Dragging towards the peak will decrease the full width at half maximum height (FWHM).



The Adjust Position and Adjust Width cursors should be used in conjunction with the Peak Searching and Fitting module - see section 4.6.7, page 134.

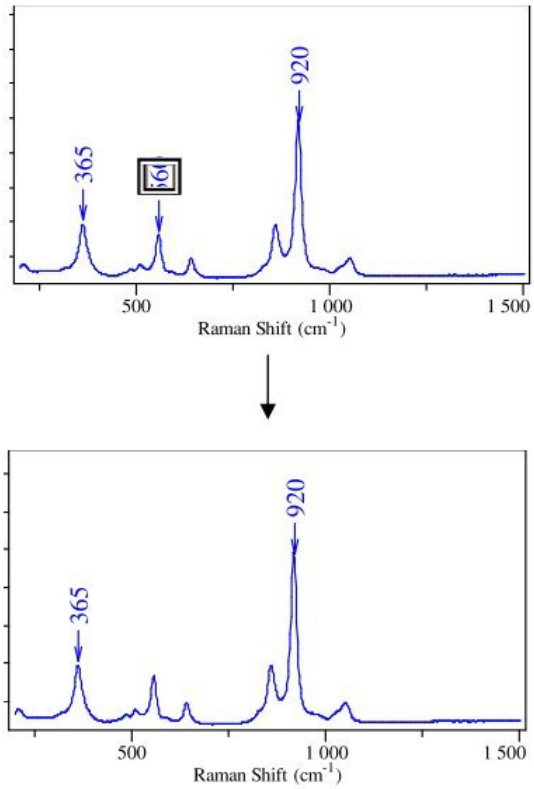
Available for: *Spectrum, SpIm, Point, Model*

5.12. Remove Peak

Activates the Remove Peak cursor, allowing a peak label to be removed from a spectrum.

When this icon is active, and mouse is hovered over a peak the mouse cursor changes to the Remove Peak cursor.

Left click on a peak label to remove it from the spectrum.



The Remove Peak cursor should be used in conjunction with the Peak Searching and Fitting module - see section 4.6.7, page 134.

Available for: *Spectrum, SpIm, Point, Model*

5.13. Integral

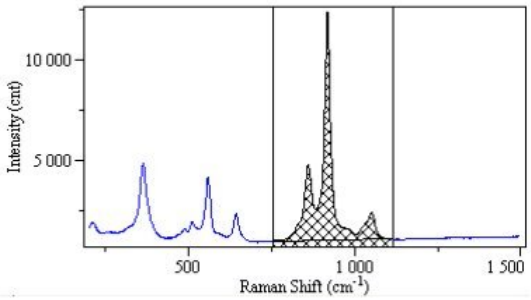
Activates the Integral cursors and opens the Integral dialog window, allowing the integrated area/sum of a region of the spectrum to be calculated.

Note that this function does not apply any deconvolution of overlapping peaks – if the area of peaks which are overlapping needs to be calculated it is necessary to perform a full peak fitting routine. See section 4.6.7, page 134 for full information about the Peak Searching and Fitting module.

Similarly, the baseline function of the Integral cursors uses a basic linear baseline – if more complex baselines are present then it is necessary to perform a full baseline subtraction routine. See section 4.6.2, page 115 for full information about the Baseline Correction module.

When this icon is active, the Integral cursors are displayed on the spectrum. In addition to the cursors the display includes

- The shaded part of the spectrum from where the area/sum value is calculated
- The baseline used for the area/sum calculation.



Left click and drag on either cursor to adjust its position. The values in the Integral dialog window will be automatically updated.

If one or both of the cursors are not visible on the spectrum, do one of the following:

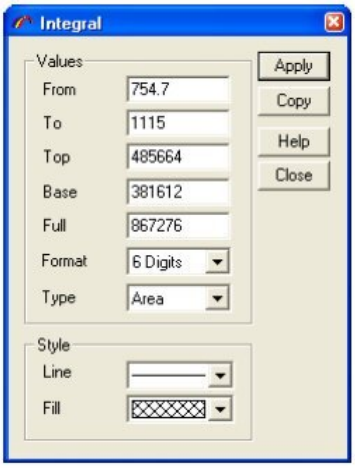
- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.

- Right click and select "Center cursor".

Available for: Spectrum, Point, Model

5.13.1. Integral Dialog Window

The Integral dialog window displays the integrated area/sum of the spectrum between the Integral cursors, and allows the information display to be configured, and the cursor positions to be manually adjusted.



From

Displays the spectral position of the lower cursor. The value can be manually adjusted by typing in the desired value and clicking **[Apply]**.

To
Displays the spectral position of the upper cursor. The value can be manually adjusted by typing in the desired value and clicking **[Apply]**.

Top
Displays the peak Sum or Area above the baseline in the shaded region. Note that **Top** = **Full** – **Base**.

Base
Displays the peak Sum or Area below the baseline in the shaded region. Note that **Base** = **Full** – **Top**.

Full
Displays the full peak Sum or Area in the shaded region. Note that **Full** = **Top** + **Base**.

Format
Click on the "Format" drop down box to select the maximum number of significant digits displayed in the "Top", "Base" and "Full" boxes.

Type
Click on the "Type" drop down box to select whether peak Area or Sum should be calculated and displayed in the "Top", "Base" and "Full" boxes.

Line
Click on the "Line" drop down box to set the color, width and line style used to outline the shaded area between the Integral cursors.

Fill
Click on the "Fill" drop down box to set the color and style used to fill the shaded area between the Integral cursors.

Apply
Click on **[Apply]** to update the cursor display on the spectrum according to values manually set in the "From" and "To" boxes.

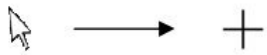
Copy
Click on **[Copy]** to copy the From, To, Top, Base and Full values to the clipboard, so that they can be pasted into other programs.

Note that Integral dialog window will be automatically closed when a different Graphical Manipulation icon is activated. If **[Close]** is used to close the Integral dialog window the Integral icon will still be active. To restore the Integral dialog window, activate a different icon, and then re-activate the Integral icon; the Integral cursors and dialog window will be displayed again.

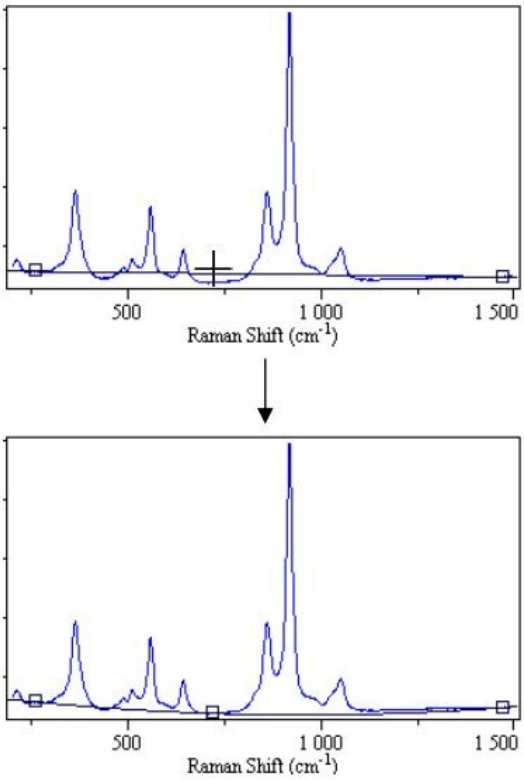
5.14. Add Baseline Points

Activates the Add Baseline Points icon, allowing baseline points to be manually added to a spectrum, prior to baseline correcting a spectrum. The type of baseline displayed when using the Add Baseline Points icon will depend on settings in the Baseline dialog window – see section 4.6.2, page 115.

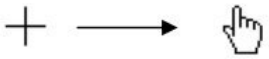
When this icon is active, the cursor will change from the mouse cursor to the Add Baseline Points cursor.



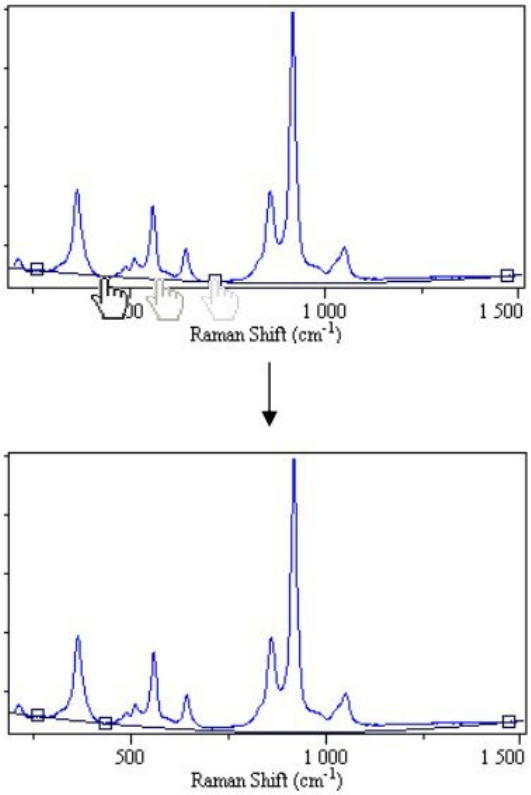
Left click the Add Baseline Points cursor on the spectrum to add a baseline point to the displayed baseline curve. If there is no baseline curve on the spectrum the first left mouse click in this mode will create the baseline.



When the Add Baseline Points cursor is active, and the mouse is hovered over an existing baseline point, the cursor changes to a Move Baseline Points cursor.



Left click the Move Baseline Points cursor to drag the baseline point to a new position.



The Add Baseline Points cursor should be used in conjunction with the Baseline Correction module - see section 4.6.2, page 115.

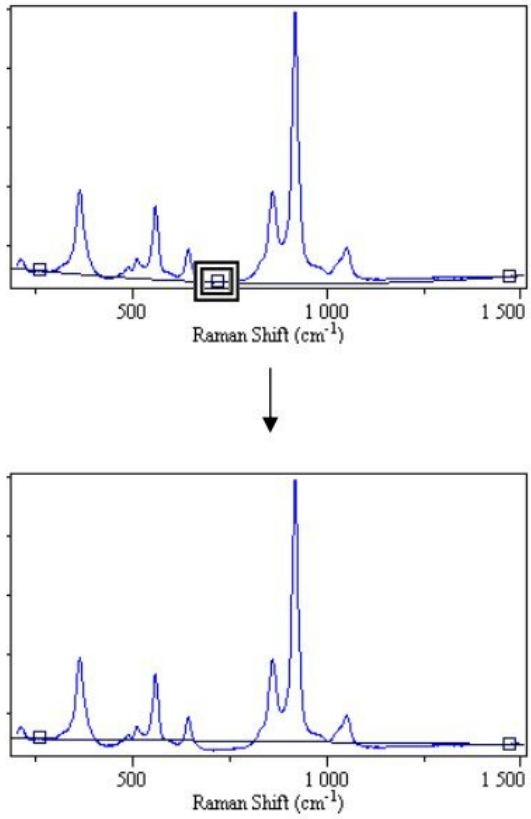
Available for: *Spectrum, SpIm, Point, Model*

5.15. Remove Baseline Points

Activates the Remove Baseline Points cursor, allowing baseline points to be manually removed from a spectrum, prior to baseline correcting a spectrum.

When this icon is active, and the mouse is hovered over an existing baseline point, the cursor changes to the Remove Baseline Points cursor.

Left click on a baseline point to remove it from the spectrum.



The Remove Baseline Points cursor should be used in conjunction with the Baseline Correction module - see section 4.6.2, page 115.

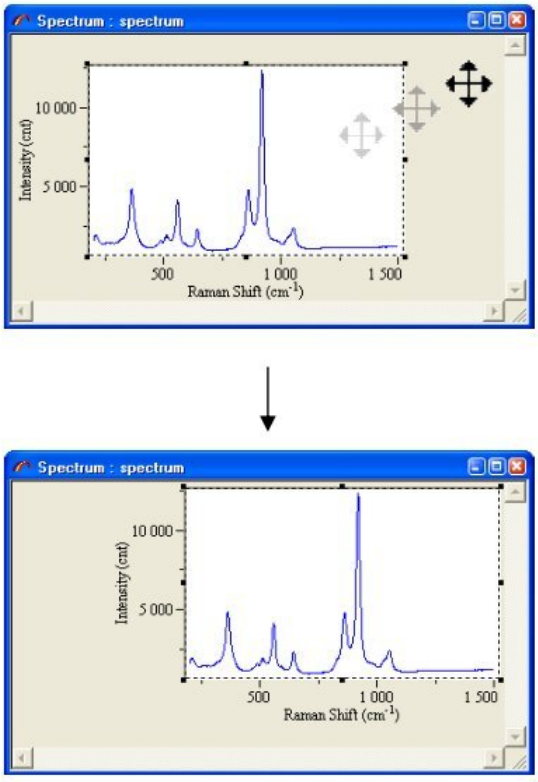
Available for: *Spectrum, SpIm, Point, Model*

5.16. Axes

Activates the Axes cursor, allowing the position and size of the spectrum/profile/image display in the active window to be adjusted.

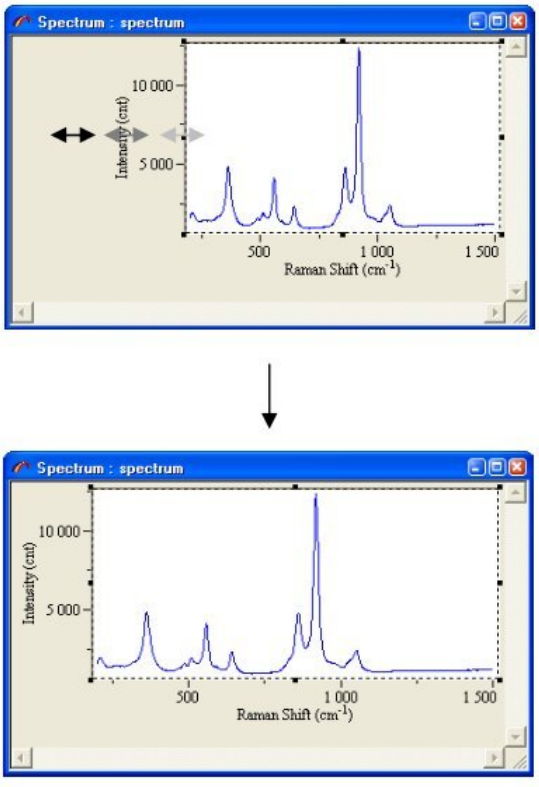
When this icon is active, and the mouse is hovered over the spectrum/profile/image display, the cursor changes to the Shift Axes cursor.

Left click and drag the Shift Axes cursor to adjust the position of the spectrum/profile/image display in the window.



When this icon is active, and the mouse is hovered over one of the axis drag points, the cursor changes to the Adjust Axes cursor.

Left click and drag the Adjust Axes cursor to adjust the spectrum/profile/image display size in the window.



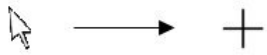
Note that only information visible within the window will be active for copy and paste functions. Thus it is important that axes titles, scales and the spectrum/profile/image display are kept within the boundary of the window.

Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

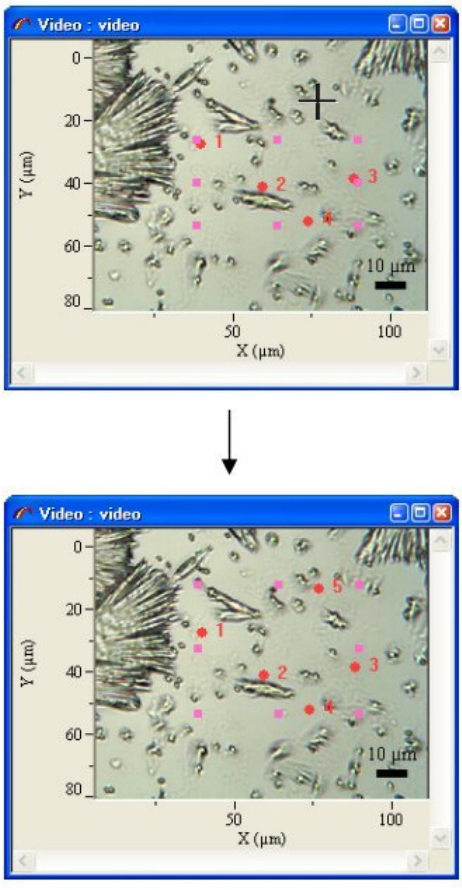
5.17. Points Mapping

Activates the Points Mapping cursor, allowing positions for an automated multipoint acquisition to be specified on the active video image.

When this icon is active, and the mouse is hovered over the video image the cursor will change from to the Add Points cursor.



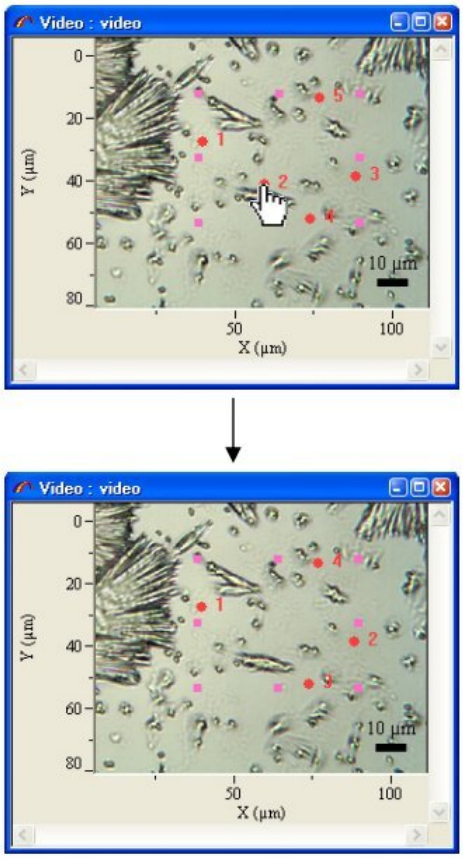
Left click with the Add Points cursor to add a multipoint position to the video image.



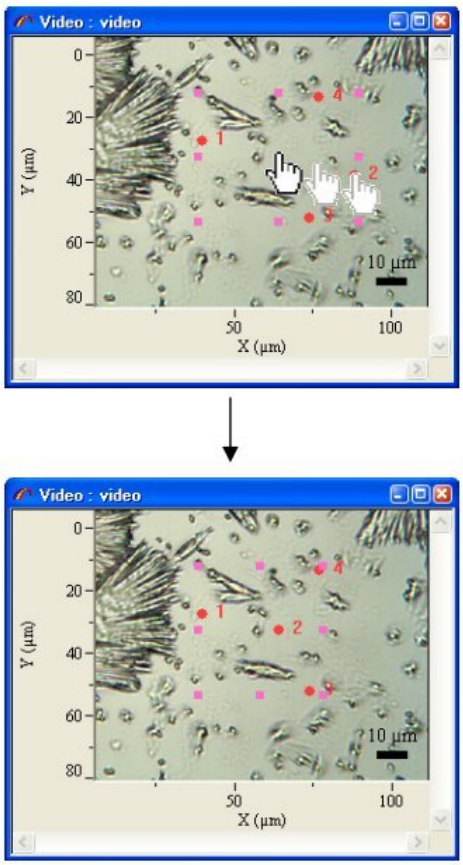
The points which are added are sequentially numbered, starting at 1. They will be analysed in the order they are added.

When the Add Points cursor is active, and the mouse is hovered over an existing multipoint position, the cursor changes to the Move/Delete Points cursor.

Left click with the Move/Delete Points cursor to delete the existing multipoint position. The other points displayed on the video will be renumbered accordingly.



Left click and drag with the Move/Delete Points cursor to move the existing multipoint position to a new position.



To clear all existing points and set point 1 at the center of the video image do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.


- Right click and select "Center cursor".

Note that the automated multipoint acquisition is started by using the Mapping Acquisition icon – see section 4.5.4, page 96. The data is acquired as a multidimensional spectral array, with the standard SpIm, Point and Map windows.


Available for: *Video*

5.18. Hor Line Mapping

Activates the Horizontal Line Mapping cursor, allowing a horizontal line for line mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the line, the cursor changes to the Shift Hor Line cursor.

Left click and drag with the Shift Hor Line cursor to move the line to the desired position on the video image.

When this icon is active, and the mouse is hovered over the line drag points (at each end of the line), the cursor changes to the Adjust Hor Line cursor.

Left click and drag with the Adjust Hor Line cursor to make the line longer or shorter.

To clear the existing line, and create a new default line at the center of the video image do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.

- Right click and select "Center cursor".

The Shift Hor Line and Adjust Hor Line cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: Video

5.19. Rectangular Mapping

Activates the Rectangular Mapping cursor, allowing a rectangular area for mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the existing rectangle, the cursor changes to the Shift Rectangle cursor.

Left click and drag with the Shift Rectangle cursor to move the line to the desired position on the video image.

When this icon is active, and the mouse is hovered over the rectangle drag points, the cursor changes to the Adjust Rectangle cursor.

Left click and drag with the Adjust Rectangle cursor to adjust the rectangle size.

To clear the existing rectangle and create a new default rectangle at the center of the video image do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.

- Right click and select "Center cursor".

The Shift Rectangle and Adjust Rectangle cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: *Video*

5.20. Line Mapping

Activates the Line Mapping cursor, allowing a line for line mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the line, the cursor changes to the Shift Line cursor.

Left click and drag with the Shift Line cursor to move the line to the desired position on the video image.

When this icon is active, and the mouse is hovered over the line drag points (at each end of the line), the cursor changes to the Adjust Line cursor.

Left click and drag with the Adjust Line cursor to make the line longer or shorter, or to rotate the line about the opposite drag point.

To clear the existing line and create a new default line at the center of the video image do one of the following:

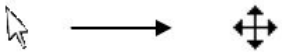
- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.  
- Right click and select "Center cursor".

The Shift Line and Adjust Line cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: *Video*

5.21. Circle Mapping

Activates the Circle Mapping cursor, allowing a circle for mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the line, the cursor changes to the Shift Circle cursor. 

Left click and drag with the Shift Circle cursor to move the line to the desired position on the video image.

When this icon is active, and the mouse is hovered over the circle area drag points (at each edge of the circle area), the cursor changes to the Adjust Circle cursor. 

Left click and drag with the Adjust Circle cursor to adjust the circle size.

To clear the existing circle and create a new default circle at the center of the video image do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.  
- Right click and select "Center cursor".

The Shift Circle and Adjust Circle cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: *Video*

5.22. Ver Line Mapping

Activates the Vertical Line Mapping cursor, allowing a vertical line for line mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the line, the cursor changes to the Shift Ver Line cursor.

Left click and drag with the Shift Ver Line cursor to move the line to the desired position on the video image.

When this icon is active, and the mouse is hovered over the line drag points (at each end of the line), the cursor changes to the Adjust Ver Line cursor.

Left click and drag with the Adjust Ver Line cursor to make the line longer or shorter.

To clear the existing line, and create a new default line at the center of the video image do one of the following:

- Click on the “Center Cursors” icon in the Icon bar– see 4.4.3, page 92.

- Right click and select “Center cursor”.

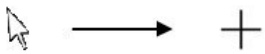
The Shift Ver Line and Adjust Ver Line cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: *Video*

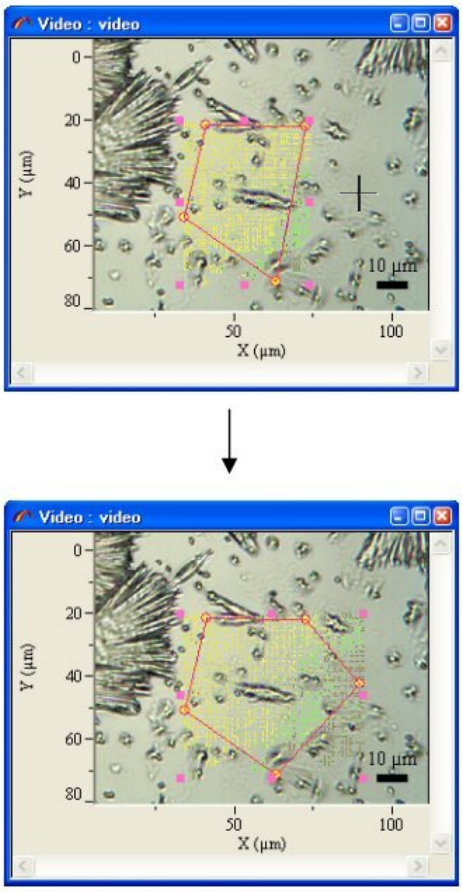
5.23. Polygon Mapping

Activates the Polygon Mapping cursor, allowing a polygon for mapping to be defined on the active video image.

When this icon is active, and the mouse is hovered over the video image the cursor will change to the Add Polygon Points cursor.

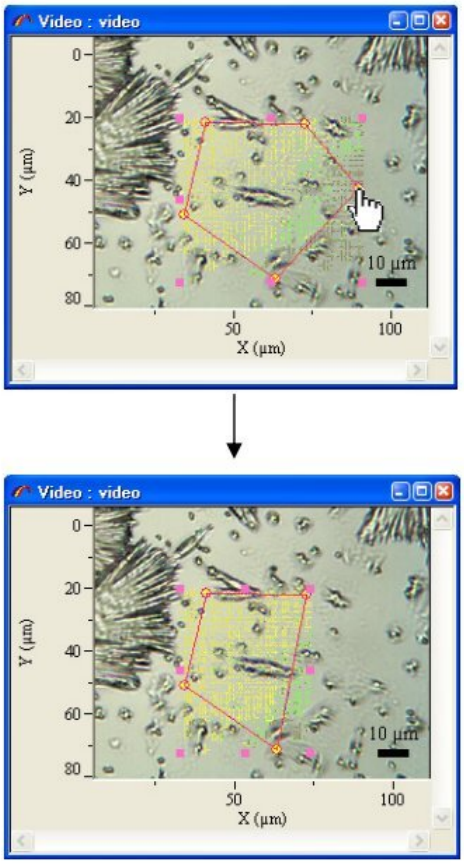


Left click with the Add Polygon Points cursor to add a polygon definition point to the video image.

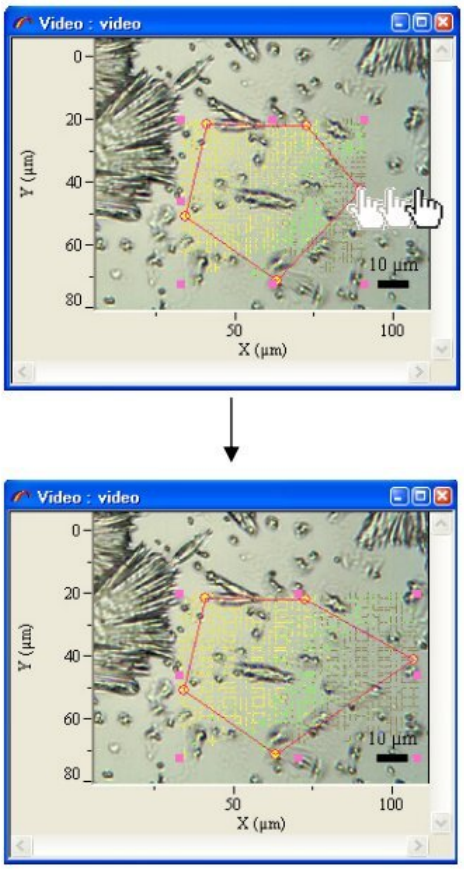


When the Add Polygon Points cursor is active, and the mouse is hovered over an existing polygon definition point, the cursor changes to the Move/Delete Polygon Points cursor.

Left click with the Move/Delete Polgyon Points cursor to delete the existing polygon definition point.



Left click and drag with the Move/Delete Polygon Points cursor to move the existing polygon definition point to a new position.



To clear all existing polygon definition points, and set a single point at the center of the video image do one of the following:

- Click on the "Center Cursors" icon in the Icon bar– see 4.4.3, page 92.

- Right click and select "Center cursor".

Note that the polygon shape is created by locating nearest neighbours to each polygon definition point. Slight adjustment of polygon definition points may be required to obtain the desired polygon mapping shape.

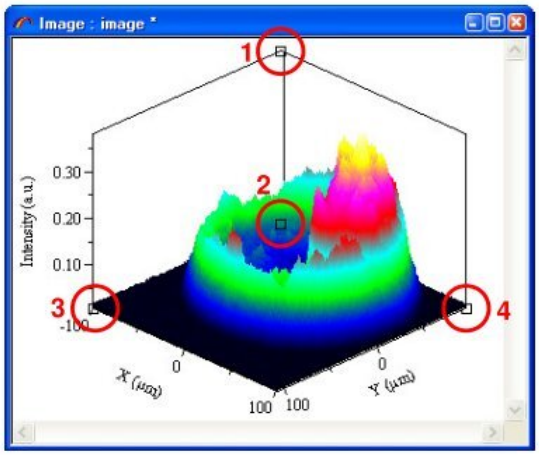
The Add Polygon Points and Move/Delete Polygon Points cursors should be used in conjunction with the Mapping Properties dialog window - see section 4.5.5, page 97.

Available for: *Video*

5.24. Axes3D

Activates axis drag points on a 3D image, allowing its position, shape and perspective to be modified.

When this icon is active, four drag points are activated on the 3D image.



Left click and drag the drag points to adjust the position, shape and perspective of the image:

Drag Point 1
Dragging up/down adjusts the size of the vertical (intensity) axis.

Dragging left/right skews the image about its center point, keeping the left hand side and right hand side vertical axes static.

Drag Point 2
Dragging up/down/left/right adjusts the image position within the display window.

Drag Point 3
Dragging up/down/left/right skews the image, keeping the back and right hand side vertical axes static.

Drag Point 4
Dragging up/down/left/right skews the image, keeping the back and left hand side vertical axes static.

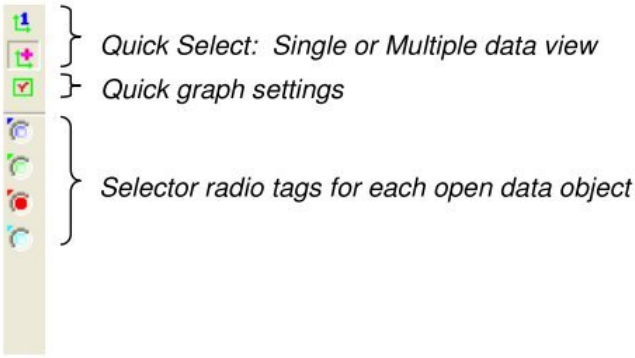
To restore the 3D image to its default display, right click and select Axes, and then click on **[Re-center image]** – see section 8.8.2, page 215.

Available for: Video, SpIm, Map, Score (when displayed in 3D mode)

6. Data Bar

The Data bar located on the right hand side of the LabSpec 5 graphical user interface (GUI) allows individual data objects (e.g., spectra, images, multidimensional spectral arrays) to be selected from a group of open objects. In addition, the Data bar allows control of quick window formatting tools.

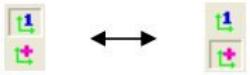
This is an active toolbar, and the number of data object selector radio tags (see section 6.3, page 198) will automatically update according to number of open objects in the currently selected window.



6.1. Quick Select: Single or Multiple Data View

The Single and Multiple Data Graph icons allow fast selection of overlay mode for the active window.

Note that when clicked an icon will be locked down. Only one icon can be active and locked down at a time.

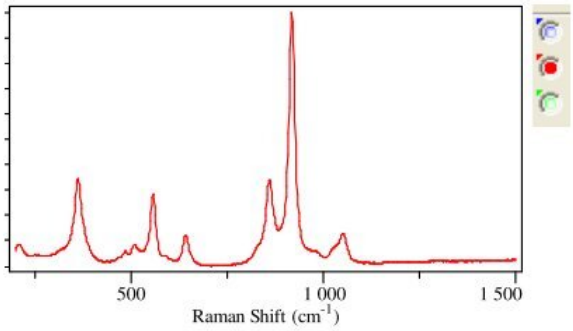


The overlay mode can also be set using the Format and Scale dialog window – see section 8.1, page 204.

6.1.1. Single Data Graph

When the Single Data Graph icon is active and locked, only the active data object will be displayed in the window. For example, if there are three spectra open, only the active spectrum will be displayed. Selecting a different spectrum using the Data bar (see section 6.3, page 198) will cause the selected spectrum to be displayed.

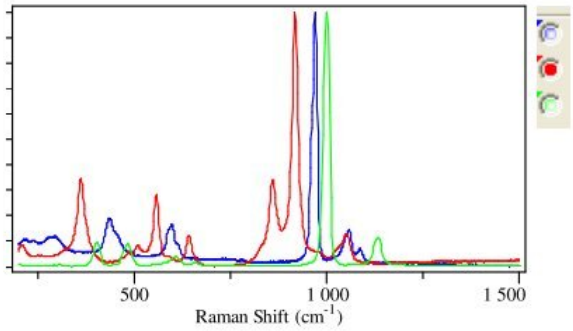
In the example shown right, there are three open spectra, but only the active spectrum (——) is displayed.



6.1.2. Multiple Data Graph

When the Multiple Data Graph icon is active and locked, all open data objects will be displayed in the window. For example, if there are three spectra open, all three will be displayed in an overlay mode in the window.

In the example shown right, there are three open spectra, all of which are displayed.

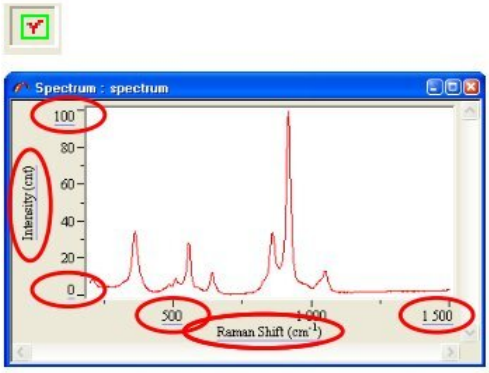


Note that the overlay mode ("Overlay", "Tile", "Stack", "Projection" or "Trace") used with the Multiple Data Graph icon will be the mode set in the Format and Scale dialog window (see section 8.1, page 204). To adjust the overlay mode right click and select "Format and Scale", and select the desired mode from the "Mode" drop down box.

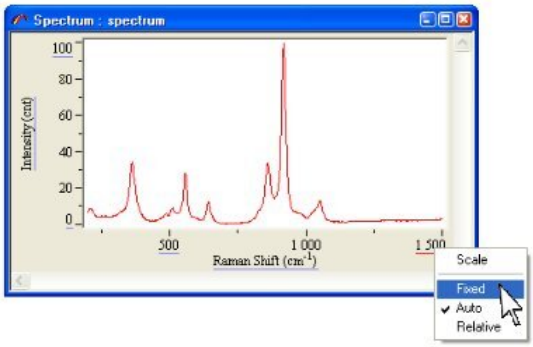
6.2. Fast Graph Settings

When the Fast Graph Settings icon is active and locked, the formatting of axis units and scaling can be quickly modified.

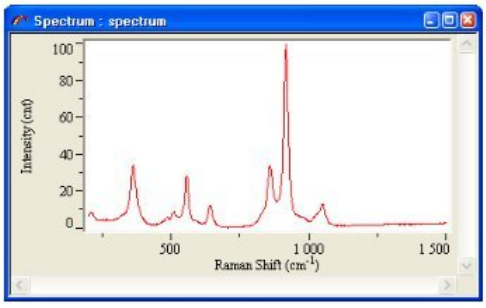
When Fast Graph Settings is active, the titles/units and maximum/minimum values on all visible axes are underlined.



Left click on an underlined item to modify its formatting, by selecting a format from the drop down list.



When Fast Graph Settings is inactive, the titles/units and minimum/maximum values on all visible axes are not underlined, and their formatting cannot be modified using a left click.



Complete formatting is possible using the Format and Scale dialog window – see section 8.1, page 204 for full information.

Note that the current status of the Fast Graph Settings (i.e., active or inactive) is also displayed in the right click menu, under “Fast Settings”. When the Fast Graph Settings is active, “Fast Settings” will be ticked.

6.3. Selector Radio Tags for Open Data Objects

When multiple data objects (e.g., spectra, images, multidimensional spectral arrays) of a similar type are open within a single display window, the Data bar will display a selector radio tag for each object.

Each radio tag will be displayed in the same colour as the data object.

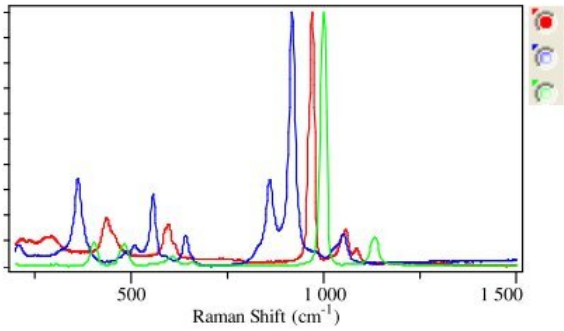
- An active object has a color-filled radio tag, as shown right.

- An inactive object has a hollow radio tag, as shown right.

Hover the mouse cursor over a radio tag to see the data object name.

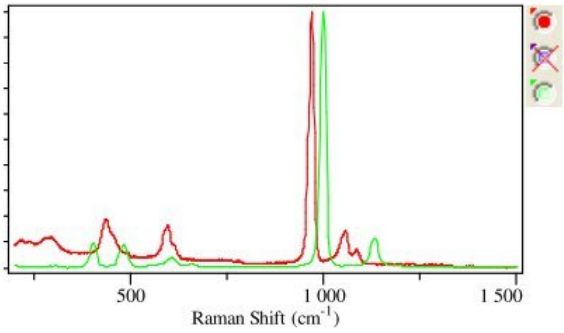
<CTRL>+Click on a radio tag to hide/show an individual object. If an object is hidden the radio tag will display a red cross.

- In the example shown right there are three open spectra, all of which are displayed.



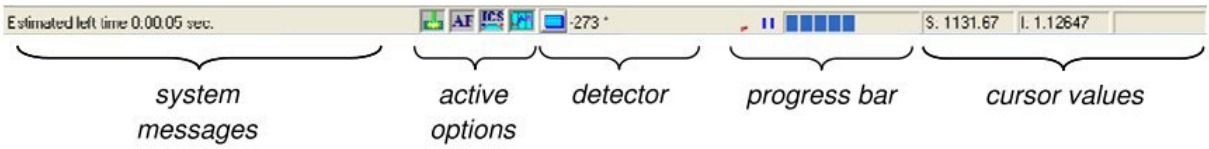
- In the example shown right there are three open spectra, but one (——) has been hidden using <CTRL>+Click and is not displayed.

  The spectrum can be shown again by <CTRL>+Clicking on its radio tag.



7. Status Bar

The Status bar displays information about system status (e.g., progress of an acquisition), active options (e.g., intensity correction, or extended range settings), and cursor values.



7.1. System Messages

System messages show information relevant to the current operation. Typical examples include the estimated time remaining until completion of a measurement, current status/operation of the autocalibration routine, or current status/operation of the spectrometer.

7.2. Active Options

The Active Options icons indicate whether certain data acquisition options are active – typically these are options which have a significant effect on the measurement.

Photo-bleaching On

When Photo-bleaching is active (i.e., the photo-bleaching time is >0s) the Photo-bleaching On icon is displayed.

For more information about Photo-bleaching and how to turn it on and off, please see section 3.5.4.4, page 35).

Autofocus On

When Autofocus is active the Autofocus On icon is displayed.

For more information about Autofocus and how to turn it on and off, please see section 3.5.4.8, page 39).

Intensity Correction On

When Intensity Correction is active the Intensity Correction On icon is displayed.

For more information about Intensity Correction and how to turn it on and off, please see section 3.5.4.14, page 43.

Extended Range On

When an Extended Range measurement is active (i.e., the extended range function is set to either Multiwindow or Autoscanning) the Extended Range On icon is displayed.

For more information about Extended Range acquisition and how to turn it on and off, please see section 3.5.6, page 49.

7.3. Detector

The Detector display shows the detector temperature (in degrees celsius, °C), and (on systems equipped with multiple detectors) can be used to switch from one detector to another.

7.3.1. Detector Temperature

The detector icon is colored according to the temperature status of the active detector, relative to the temperature set in the "Detector" dialog window (see section 3.5.7, page 57).

- Actual temperature is between set temperature and 5°C above the set temperature; detector can be used.

- Actual temperature is between 5°C and 10°C above the set temperature.

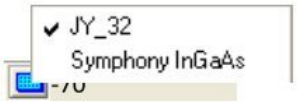
  Detector can be used, but data acquired in this state may exhibit slightly reduced quality (signal to noise).

- Actual temperature is greater than 10°C above the set temperature; it is recommended that the detector is not used.

  Note that use of a detector at high temperature will not damage it, but data acquired in this state will exhibit significantly reduced quality (signal to noise).

7.3.2. Switching Detector

On systems equipped with multiple detectors, click on the detector icon to view a list of available detectors. The currently active detector is indicated with a tick.

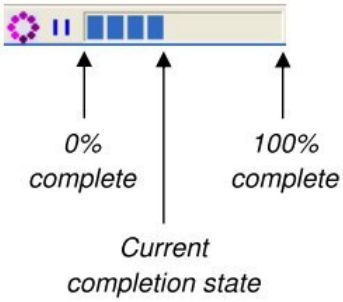


To switch detector, select the desired detector from the list of available detectors. Note that a detector initialization message may be displayed when the detector is switched.

7.4. Progress Bar

The Progress Bar indicates whether a hardware/software process is active, and displays the approximate progress of an active process. It is also possible to temporarily pause a process using the Progress Bar "Pause" icon.

When a process is active, the Progress Bar displays a moving circle icon, and blue bars which indicate the current progress.



Left click on the "Pause" icon to pause the current process. The process will continue until the current section of the process is completed, and it will then pause. The "Pause" icon will change into the "Start" icon.

Left click on the "Start" icon to re-start a paused process. The "Start" icon will change into the "Pause" icon.

When no process is active, the Progress Bar is empty.

7.5. Cursor Values

The Cursor Value display shows a range of information depending on the type of cursor which is active – e.g., the X and Y axis positions of the cursors, the spectrum/profile/image intensity at the cursor position, width between two cursors, and approximate peak width.

The following symbols are used to identify information displayed in the Cursor Value display.

- S – spectral (X axis) position in a spectrum. The units will be those selected in Options > Unit (see section 3.4.1, page 22), typically Raman shift (cm^{-1}) or wavelength (nm).
- I – intensity. The units for spectra and cursor generated profiles/maps will be those selected in Options > Unit (see section 3.4.1, page 22), typically counts (cnt) or counts per second (cnt/s). The units for video images will be arbitrary units (a.u.).
- X, Y, Z – spatial position in a profile or image. The units will be micrometers (µm).
- P – spectral (X axis) width between a paired set of map analysis cursors. The units will be those selected in Options > Unit (see section 3.4.1, page 22), typically Raman shift (cm^{-1}) or wavelength (nm).
- W – approximate peak width as indicated by the Peak cursor. The units will be those selected in Options > Unit (see section 3.4.1, page 22), typically Raman shift (cm^{-1}) or wavelength (nm).

8. Right Click Menus

Right mouse clicking on the data windows will display a menu, which typically will allow access to additional dialog windows, provides shortcuts to other functions within LabSpec 5, or provides information about the current configuration.

The right click menu is active, and its appearance and content will update according to the currently selected window. For example, the options appearing for a Spectrum window will differ from those appearing for a video image.

At the end of each function's description, a list of windows where the function is available in the right click menu is given. The possible windows are as follows:

Spectrum
The spectrum display window for individual spectra acquired using the real time display (RTD) acquisition (/) and spectrum acquisition (/) modes.

Video
The video display window for optical images acquired with the integrated microscope camera(s).

SpIm
The overlay of all spectra within a multidimensional spectral array.

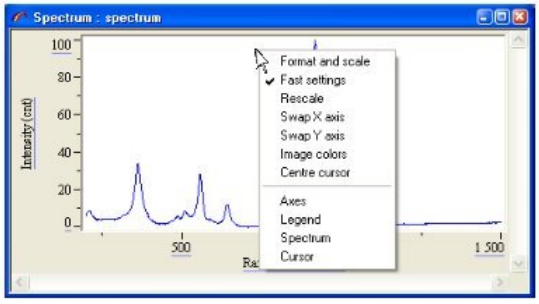
Point
The spectrum at the current cursor position within a multidimensional spectral array.

Map
The cursor intensity profile/image display created from a multidimensional spectral array.

Score
The score profile/image created by DCLS modelling of a multidimensional spectral array

Model
The reference component spectra used for DCLS modelling of a multidimensional spectral array.

To display the right click menu, right click anywhere within the active window. Left click on a menu item to open a new dialog window, or run the function.

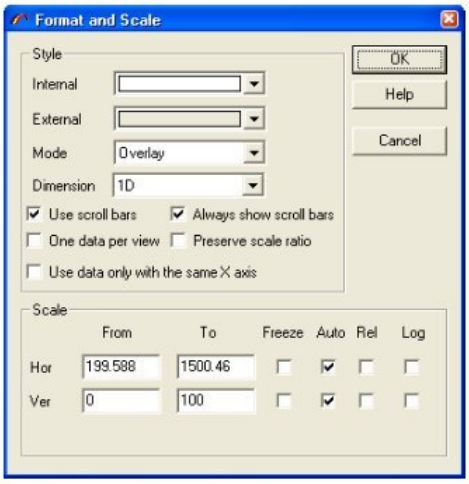


Note that setting a function in a right click menu dialog window will only set that function for the active window. It is possible to have different settings for different windows. For example, a Spectrum and Point window can have different format and scale settings, by applying different settings through the Format and Scale dialog window for each window.

8.1. Format and Scale

The Format and Scale dialog window allows control over the window display format, and the data scaling within the window.

Note that depending on the data window selected, the Format and Scale dialog window may slightly differ from that shown below.



Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.1.1. Style

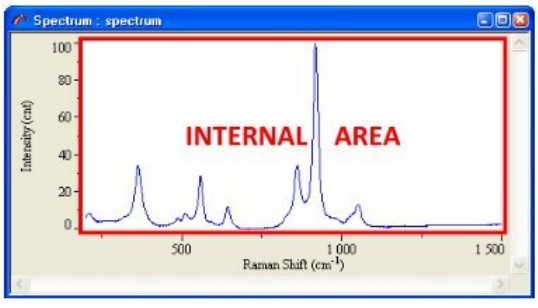
The Style section of the Format and Scale dialog window allows control over the appearance of the data window, and how data is displayed within it.

Internal

Select the internal area color and fill option from the "Internal" color drop down box.

Note that when a data window is copied as an image, it will be copied with the default internal white background.

The internal area is shaded in the image shown right.

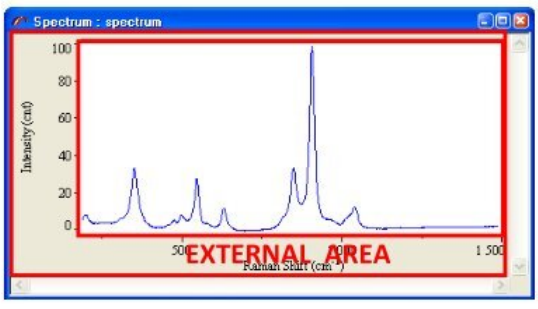


External

Select the external area color and fill option from the "External" color drop down box.

Note that when a data window is copied as an image, it will be copied with the default external white background.

The external area is shaded in the image shown right.

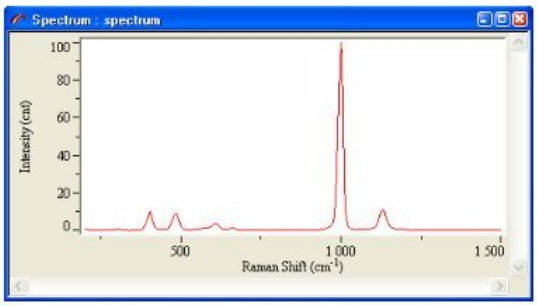


Mode

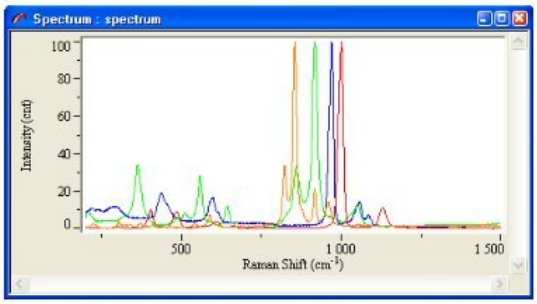
Select the overlay mode for data objects (e.g., spectra, images, multidimensional spectral arrays) from the "Mode" drop down box.

A number of overlay modes are available:

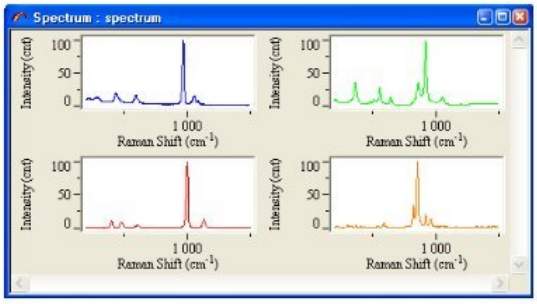
- Single – display only the currently active data object in the window.



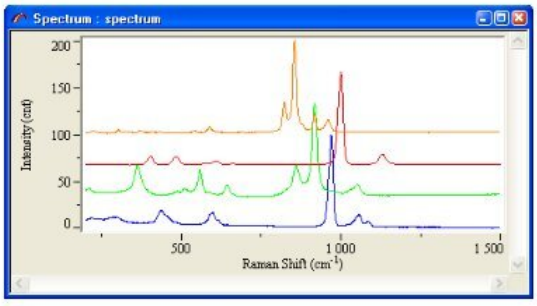
- Overlay – display all open data objects in the window.



- Tile – display all open data objects in separate tiled sections within the window.

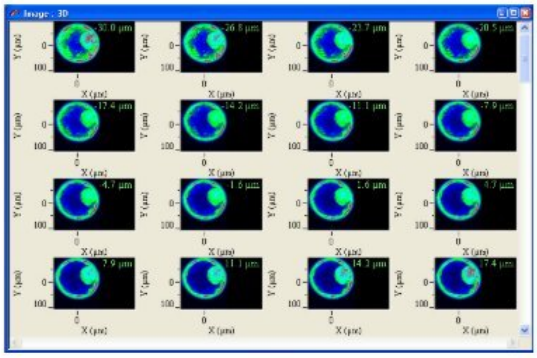


- Stack – display all open data objects in the window, offsetting each by a certain amount.



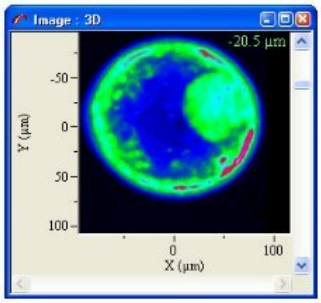
- Projection – displays a series of images corresponding to the open data object in separate tiled sections within the window.

  This mode is only available for multidimensional spectral arrays, and is intended for display of 3D data sets (e.g., XYZ datacubes).



- Trace - displays a single image from a multi-image single data object within the window. The scroll bar on the right hand side can be used to scroll through each image corresponding to the data object; the axis value for the image is displayed in the top right hand corner of the window.

  This mode is only available for multidimensional spectral arrays, and is intended for display of 3D data sets (e.g., XYZ datacubes).

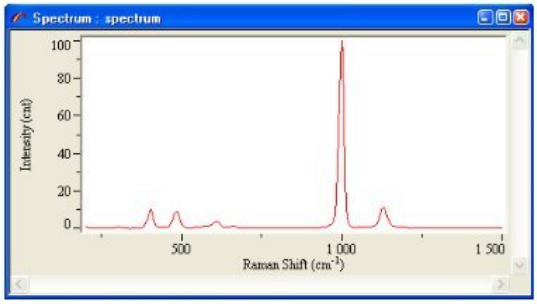


Dimension

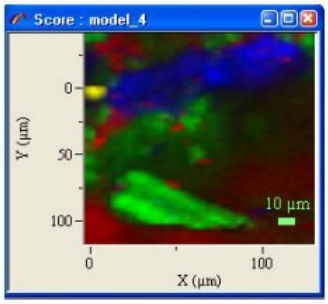
Select the display dimension from the "Dimension" drop down box. The number of dimensions used for data display refers to the number of non-intensity axes. For example, a simple spectrum is considered a 1D display, because it constitutes the intensity dimension plus one other dimension (the spectral axis, typically Raman shift or wavelength). A video image is considered a 2D display, because it constitutes the intensity dimension plus two spatial axes (X and Y).

Three display dimensions are available:

- 1D – simple 'graph' presentation, typically used for spectra (including Spectrum, SpIm, Point and Model windows).

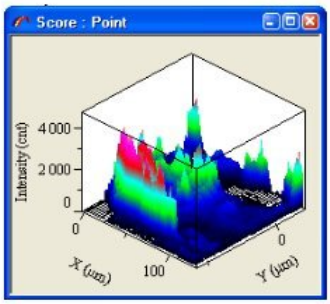


- 2D – simple image presentation, typically used for images (including video, map and score windows). Multiple images can be overlaid in this mode.



- 3D – for pseudo 3D representation of 2D data, where all three axes (intensity plus two spatial) are visible. Only a single image component can be displayed in this mode.

  Note that 3D images are configured using the right click menus Axes (see section 8.8.2, page 215) and 3Dimage (see section 8.18, page 224).



Use Scroll Bars

When "Use scroll bars" is ticked, scroll bars will be displayed if part of the data is off scale (e.g., not actually displayed in the data window).

Always Show Scroll Bars

When "Always show scroll bars" is ticked, the scroll bar display section of the data window will always be present, even if all data is displayed within the window. The scroll bar(s) will only become active if part of the data is off scale (e.g., not actually displayed in the data window).

One Data per View

When "One data per view" is ticked, each data object associated with the data window will always open into a new data window. In this case, it is not possible to overlay objects in a single window.

Preserve Scale Ratio

When "Preserve scale ratio" is ticked, the aspect ratio will be retained. Typically this function is used for image display only.

Use Data Only with Same X Axis

When "Use data only with same X axis" is ticked, only data objects which share the same X axis units will be opened within the window. Objects with other X axis units will be opened into a new data window. For example, if a spectrum with Raman shift (cm^{-1}) units is already open in a data window, when a spectrum with wavelength (nm) units is opened, a new data window will be created for it.

8.1.2. Scale

The Scale section of the Format and Scale dialog window allows control over the scaling of data within the data window.

Each available axis for the active data object is displayed in the "Scale" section, with a number of options for its scaling. Typical axes displayed here are:

- Hor – the X axis, typically the spectral axis for spectra, or the X (µm) axis for images.
- Ver – the Y axis, typically the intensity axis for spectra, or the Y (µm) axis for images.
- Intens – the intensity axis for images.

From and ***To***
Displays the start ("From") and stop ("To") axis values which will be displayed. These can be manually adjusted by typing in desired values and clicking **[OK]**.

Freeze
When "Freeze" is ticked the axis scaling is fixed, and will not be affected by scale normalization (see section 4.4.1, page 91), its right click shortcut "Rescale" or its keyboard shortcut <CTRL>+N.

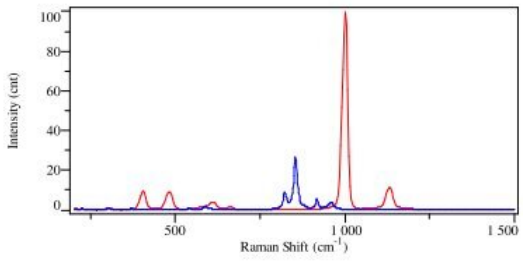
The axis scaling can also be fixed using the Fast Graph Settings and selecting "Fixed" for the axis (see section 6.2, page 197).

Auto
When "Auto" is ticked the axis will autoscale to display all data open in the data window.

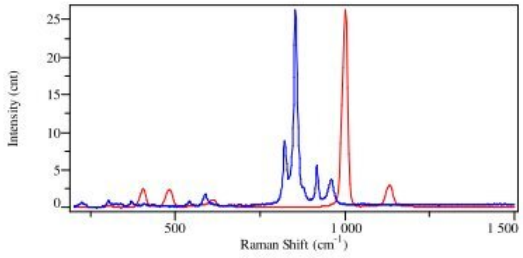
The axis scaling can also be set to autoscaling by using the Fast Graph Settings and selecting "Auto" for the axis (see section 6.2, page 197).

Rel
When "Rel" is ticked all data open in the data window will be displayed with identical minimum and maximum intensities. The scale values displayed for the axis will correspond to the active data object.

The data shown right are displayed with normal scaling.



The data shown right are displayed with relative scaling. The active spectrum is ——.



The axis scaling can also be set to relative scaling by using the Fast Graph Settings and selecting "Relative" for the axis (see section 6.2, page 197).

Log
When "Log" is ticked the axis will be displayed with logarithmic scaling rather than linear scaling.

8.2. Fast Settings

When "Fast settings" is ticked, the Fast Graph Settings are active (see section 6.2, page 197).

Left click on "Fast settings" to tick (activate) or untick (deactivate) this function.

Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.3. Rescale

Left click on "Rescale" to rescale the active window so that all data is visible. In normal operation all axes are affected, but note that an axis with scaling set to "Fixed" will not be rescaled. To rescale an axis ensure that "Freeze" or "Fixed" scaling is turned off (see section 8.1, page 204).

This function can also be activated with the Scale Normalization icon (see section 4.4.1, page 91) or with the <CTRL>+N keyboard shortcut.

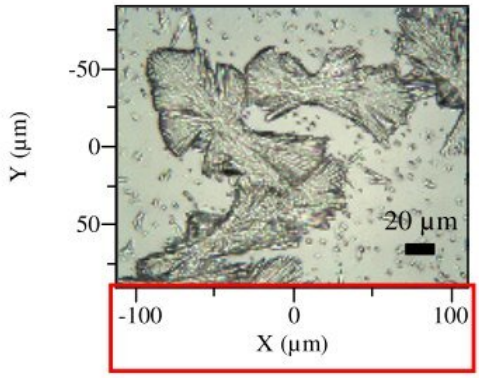
Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.4. Swap X axis

Click on "Swap X axis" to reverse the X axis display.

CAUTION: WHEN AN XY MOTORIZED SAMPLE STAGE IS PRESENT, THE AXIS DISPLAY FOR VIDEO IMAGES SHOULD ALWAYS BE LEFT IN THE DEFAULT CONFIGURATION TO ENSURE CORRECT MAPPING AND EXTENDED VIDEO IMAGING ACQUISITION.

THE X AXIS SHOULD HAVE UNITS RUNNING FROM NEGATIVE ON THE LEFT HAND SIDE, THROUGH TO POSITIVE ON THE RIGHT HAND SIDE, AS DISPLAYED RIGHT.



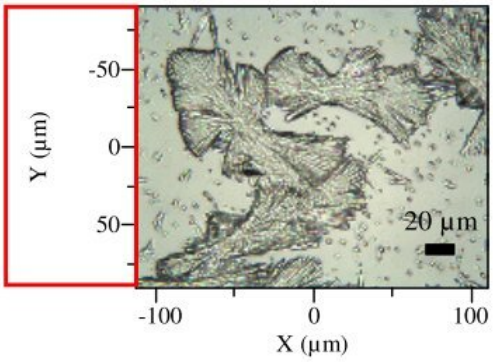
Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.5. Swap Y axis

Click on "Swap Y axis" to reverse the Y axis display.

CAUTION: WHEN AN XY MOTORIZED SAMPLE STAGE IS PRESENT, THE AXIS DISPLAY FOR VIDEO IMAGES SHOULD ALWAYS BE LEFT IN THE DEFAULT CONFIGURATION TO ENSURE CORRECT MAPPING AND EXTENDED VIDEO IMAGING ACQUISITION.

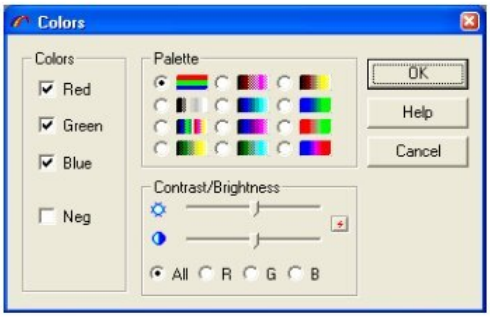
THE Y AXIS SHOULD HAVE UNITS RUNNING FROM NEGATIVE AT THE TOP, THROUGH TO POSITIVE AT THE BOTTOM, AS DISPLAYED RIGHT.



Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.6. Image Colors

The Colors dialog window allows control of the color palettes used for image rendition, and adjustment of image brightness and contrast.



Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

8.6.1. Colors

LabSpec 5 uses an RGB palette for its image rendition – by default, red (R), green (G) and blue (B) components are used to create the colored images displayed.

When a color "Red", "Green" or "Blue" is ticked, it will be used to create the colored images. For correct color rendering of images based on the selected color palette (see section 8.6.2, page 211) ensure that all three colors ("Red", "Green" and "Blue") are ticked.

When "Neg" is ticked, the negative image will be displayed for the selected color palette.

8.6.2. Palette

Available color palettes are listed in this section. Left click on one of the palette radio buttons ( ) to select the palette.

There are two types of palette:

- True color – with this palette each color component is displayed in its true color.

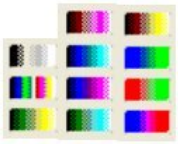
  In the case of a video image, the three components (red, green and blue) are displayed in their individual colors to yield a composite colored image.

  In the case of a map image created from a multidimensional spectral array the cursor intensity image corresponds to the cursor color. For example, the image generated using the red map analysis cursor will be displayed in a one color red scale.

  In the case of a score image created from a multidimensional spectral array the score image corresponds to the reference component spectrum displayed in the Model window. For example, the score image for a red spectrum in the Model window will be displayed in a one color red scale.

  This palette should be used for video images, and map/score images where multiple components are overlaid.

- False color – with one of these palettes, image pixels are displayed in different colors depending on their intensity.



These palettes are useful when an image has regions of both high and low intensity, which can be hard to visualize with a traditional true color palette.

These palettes should only be used for representation of a single component (e.g., a single cursor intensity map image, or a single score image).

8.6.3. Contrast and Brightness

Left click and drag the "Brightness" slider to adjust the brightness for the image display.

Left click and drag the "Contrast" slider to adjust the contrast for the image display.

If {All} is selected ( ), the brightness and contrast adjustments will affect the complete RGB palette.

If one of {R}, {G} or {B} is selected ( ), the brightness and contrast adjustments will only affect the selected component (R, red; G, green; B, blue) of the RGB palette.

Click on the Contrast and Brightness Initialization icon to reset the brightness and contrast settings to their default values. Note that this operation needs to be done individually for {All}, {R}, {G} and {B}.

8.7. Center Cursor

Left click on "Center cursor" to center the active cursor(s) in the active window.

This function is useful when cursors are not visible in a window, because their position lies outside the range of the window. Right click and select "Center cursor" and the cursor(s) will be immediately visible in the center of the window.

This function can also be activated with the Center Cursors icon (see section 4.4.3, page 92).

Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

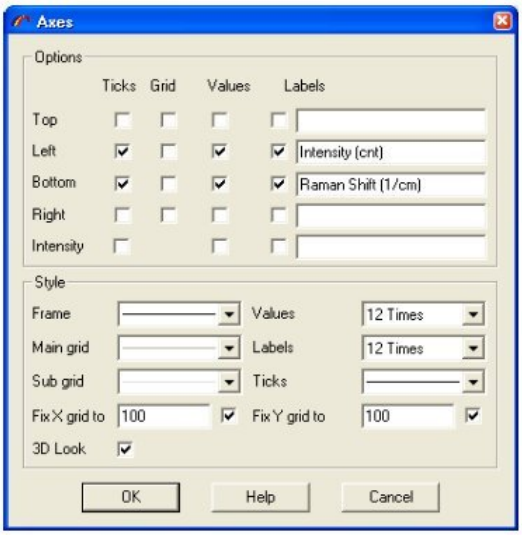
8.8. Axes

The Axes dialog window allows control over what axes are displayed, and how they are displayed within the data window.

There are two Axes dialog windows, depending on the selected dimension (see section 8.1.1, page 204) of the data window. These two windows are discussed in turn below.

Available for: Spectrum, Video, SpIm, Point, Map, Score, Model

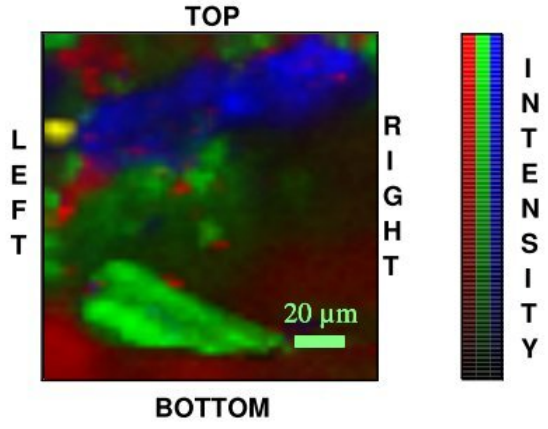
8.8.1. Axes Dialog Window for 1D and 2D Windows



8.8.1.1. Options

The five possible display axes are listed, with tick boxes to select whether ticks, grid lines, axis values and labels are shown.

The fives axes are indicated on the image shown right.



Ticks
When the "Ticks" box is ticked tick marks are displayed on the axis.

Grid
When the "Grid" box is ticked 'main' and 'sub' grid lines are displayed in the spectrum window. The spacing of the grid lines are automatically calculated by LabSpec 5, but can be manually assigned using the "Fix X grid to" and "Fix Y grid to" options in the Style section of the Axes dialog window (see section 8.8.1.2, page 214)

Values
When the "Values" box is ticked axis values are displayed along the axis.

Labels
When the "Labels" box is ticked the axis title and units are displayed adjacent to the axis. Custom labels can be used by typing in the desired axis label in the "Labels" text box.

To restore the default labels for an axis delete the text in the box.

8.8.1.2. Style

The Style section allows the formatting of the axis components (frame, ticks, grid lines, values and labels) to be set.

Frame
Select the line style for the axes frame using the "Frame" drop down box.

Main grid
Select the line style for the 'main' grid lines using the "Main grid" drop down box.

Sub grid
Select the line style for the 'sub' grid lines using the "Sub grid" drop down box.

Fix X grid to
When the "Fix X grid to" box is ticked the main grid lines for the X axis will be spaced at the interval displayed in the box. To adjust the spacing type the desired grid line spacing into the text box.

3D look
When the "3D look" box is ticked the axes frame is displayed in a "3D" style.

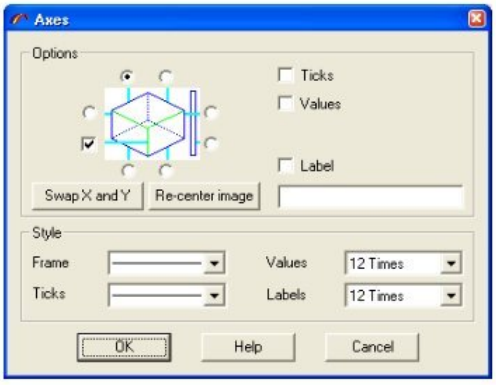
Values
Select the font style for the axis values from the "Values" drop down box.

Labels
Select the font style for the axis values from the "Labels" drop down box.

Ticks
Select the line style for the ticks using the "Ticks" drop down box.

Fix Y grid to
When the "Fix Y grid to" box is ticked the main grid lines for the Y axis will be spaced at the interval displayed in the box. To adjust the spacing type the desired grid line spacing into the text box.

8.8.2. Axes dialog Window for 3D Windows



8.8.2.1. Options

The schematic in the Options section illustrates the seven available axes (six surrounding the 3D image, and one for intensity). Each axis is configured individually.

Select an axis by clicking the appropriate radio tag. 

When the tick box is ticked the three forward axes are displayed, in front of the 3D image.

Ticks
When the "Ticks" box is ticked tick marks are displayed on the axis.

Values
When the "Values" box is ticked axis values are displayed along the axis.

Labels
When the "Labels" box is ticked the axis title and units are displayed adjacent to the axis. Custom labels can be used by typing in the desired axis label in the "Labels" text box.

To restore the default labels for an axis delete the text in the box.

Swap X and Y
Click on **[Swap X and Y]** to reverse the positions of the X and Y axes in the 3D image display.

Re-center image
Click on **[Re-center image]** to return the image to its default position in the display window. This function is useful if the image position has been modified using the Axes3D icon – see section 5.24, page 193.

8.8.2.2. Style

The Style section allows the formatting of the axis components (frame, ticks, values and labels) to be set.

Frame
Select the line style for the axes frame using the "Frame" drop down box.

Ticks
Select the line style for the ticks using the "Ticks" drop down box.

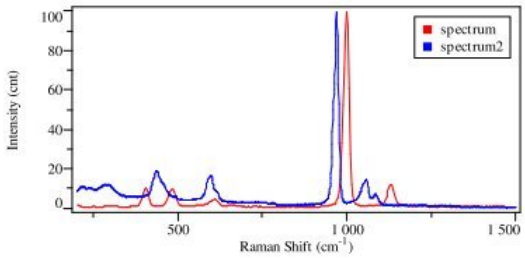
Values
Select the font style for the axis values from the "Values" drop down box.

Labels
Select the font style for the axis values from the "Labels" drop down box.

8.9. Legend

The Legend dialog window allows control of the legend display and formatting.

The legend is displayed for spectral windows, and shows the data object name and its display color.



Show
Tick the boxes for Single and Multi to activate the legend for single and overlay display modes respectively.

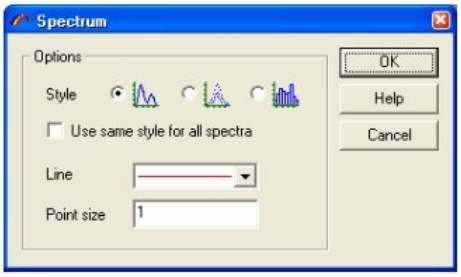
Frame
Select the line style for the legend frame using the "Frame" drop down box.

Text
Select the font style for the legend text from the "Text" drop down box.

Available for: *Spectrum, SpIm, Point, Model*

8.10. Spectrum

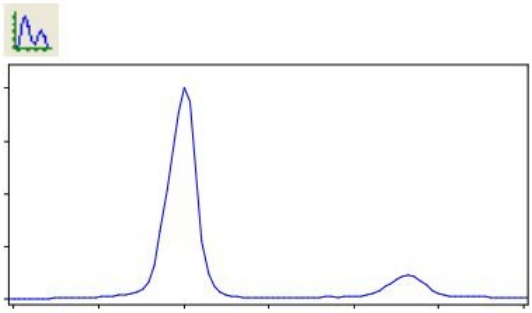
The Spectrum dialog window allows control of the spectrum display format.



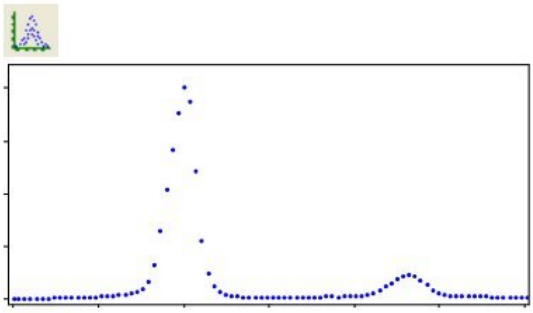
Style

Select the display style from the following options:

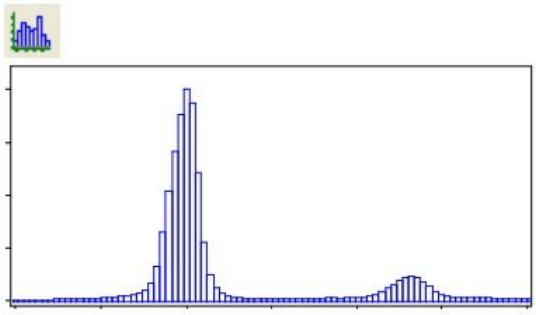
- Line



- Dot



- Bar



Use same style for all spectra
When "Use same style for all spectra" is ticked all spectra in the current window will share a common line style, based upon the current setting in the Spectrum dialog window.

If "Use same style for all spectra" is not ticked then the line style setting in the Spectrum dialog window is applied to the active spectrum only. In this mode it is possible to have multiple spectra displayed with different line styles.

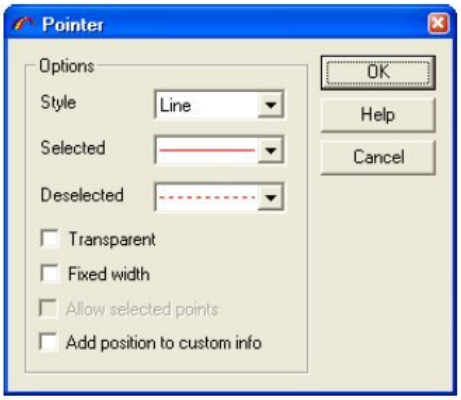
Line
Select the spectrum display color and (where appropriate) line style for the spectrum using the "Line" drop down box.

Point size
Select the point size for the "Dot" spectrum display. Note that the point color is set in the "Line" drop down box.

Available for: Spectrum, SpIm, Point, Model

8.11. Cursor

The Cursor dialog window allows control of the cursor behaviour and display format.



Style
Select the cursor style from the "Style" drop down box. The following cursor styles are available:

Spectrum, Model windows
- Line – single vertical line cursor, displaying the X axis position (S) of the cursor and the intensity (I) of the spectrum at the cursor position.
- Cross – cross hair cursor, displaying the X axis position (S) and Y axis position (I) of the cursor.
- Level – cross hair cursor which tracks the intensity of the spectrum, displaying the X axis position (S) and Y axis position (I) of the cursor. In this case, the Y axis position is equivalent to the spectrum intensity at the cursor position.
- Double – two vertical cursors, displaying the X axis position (S) of each cursor and the width between the two cursors (W).
- Peak – three linked vertical cursors, the central one locking to the maximum intensity pixel in a peak, and the outer two locking to the pixels closest to the full width at half maximum

height (FWHM) of the peak. The Peak cursor displays the X axis position (S), intensity (I) and approximate full width at half maximum height (W) of the peak at the cursor position.

Video, Map, Score windows

- Cross – cross hair cursor, displaying the X axis position (X) and Y axis position (Y) of the cursor, and pixel intensity (I) at the cursor position. For the Map and Score windows, the spectrum associated with the cursor position will be displayed in the Point window.
- Rect – rectangular cursor (resizeable by left clicking and dragging the drag points), displaying the X axis position (X) and Y axis position (Y) of the bottom, right hand corner of the rectangular cursor. For the Map and Score windows, the average spectrum from within the rectangle is displayed in the Point window.

Selected

Select the display formatting for the cursor when it is selected (i.e., active), using the Selected drop down box.

Deselected

Select the display formatting for the cursor when it is not selected (i.e., inactive), using the Deselected drop down box.

Transparent

When "Transparent" is ticked the cursor is displayed in a transparent mode, allowing features behind the cursor to be visualized.

Fixed width

When "Fixed width" is ticked the width between "Double" cursors is fixed to its current value. In this mode, both cursors will move together when dragged by the mouse. To adjust the width of the "Double" cursors ensure that "Fixed width" is unticked.

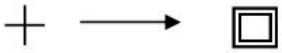
Allow selected points

When "Allow selected points" is ticked multiple discrete points within a map or score profile/image can be selected.

When <CTRL> is held down the mouse cursor changes to the Select Point cursor.

<CTRL>+click to select a position in the map or score profile/image. Multiple positions can be simultaneously selected by clicking additional positions.

When <CTRL> is held down and the mouse cursor is hovered over an existing selected point, the mouse cursor changes to the Remove point cursor.



<CTRL>+click to delete an existing selected point.

The average spectrum from the selected points will be displayed in the Point window.

Add position to custom info
When "Add position to custom info" is ticked the cursor values are included within the Custom tab of the parameters information dialog window (see section 4.4.5, page 92).

Available for: *Spectrum, Video, Map, Score, Model*

8.12. Red Cursor

The Red Cursor dialog window allows control of the cursor behaviour and display format for the red (SpImRed) map analysis cursor (see section 5.2, page 165).

The Red Cursor dialog window is identical to the standard Cursor dialog window described above (see section 8.11, page 218).

The map analysis cursor should be operated in "Double" mode. It is advisable that the color is retained as red to avoid confusion.

Available for: *SpIm, Point*

8.13. Green Cursor

The Green Cursor dialog window allows control of the cursor behaviour and display format for the green (SpImGreen) map analysis cursor (see section 5.2, page 165).

The Green Cursor dialog window is identical to the standard Cursor dialog window described above (see section 8.11, page 218).

The map analysis cursor should be operated in "Double" mode. It is advisable that the color is retained as green to avoid confusion.

Available for: *SpIm, Point*

8.14. Blue Cursor

The Blue Cursor dialog window allows control of the cursor behaviour and display format for the blue (SpImBlue) map analysis cursor (see section 5.2, page 165).

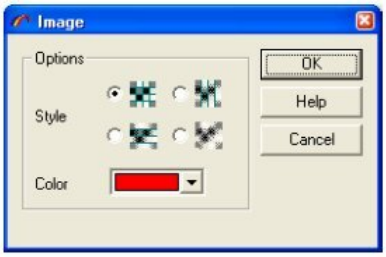
The Blue Cursor dialog window is identical to the standard Cursor dialog window described above (see section 8.11, page 218).

The map analysis cursor should be operated in "Double" mode. It is advisable that the color is retained as blue to avoid confusion.

Available for: *SpIm, Point*

8.15. Image

The Image dialog window allows the image color and smoothing options to be set.



Style
Select the smoothing interpolation style from the available options:

- No smoothing – image is displayed in raw pixelated form. 
- X smoothing – image is displayed with smoothing interpolation in the X dimension. 
- Y smoothing – image is displayed with smoothing interpolation in the Y dimension. 
- XY smoothing – image is displayed with smoothing interpolation in the X and Y dimensions. 

Color
Select the image display color for the active image component from the "Color" drop down box.

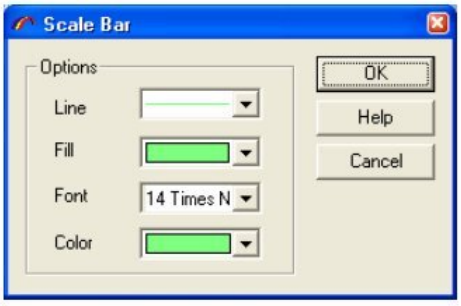
Note that the color selected is only displayed when the image is displayed in True Color mode – see section 8.6, page 210) for full information about image colors.

When the color is changed for a DCLS modelling score image the reference component spectrum display in the Model and Point windows will be automatically updated. Similarly, the score image color will be automatically updated if the reference component spectrum color is modified in the Model window.

Available for: Video, Map, Score

8.16. Scale Bar

The Scale Bar dialog window allows control of display formatting for the image scale bar.



Line
Select the line style from the "Line" drop down box for the line surrounding the scale bar.

Fill
Select the fill colour from the "Fill" drop down box for the scale bar.

Font
Select the font style from the "Font" drop down box for the scale display text. Note that the color of the text is set through the "Color" drop down box.

Color
Select the color from the "Color" drop down box for the scale display text.

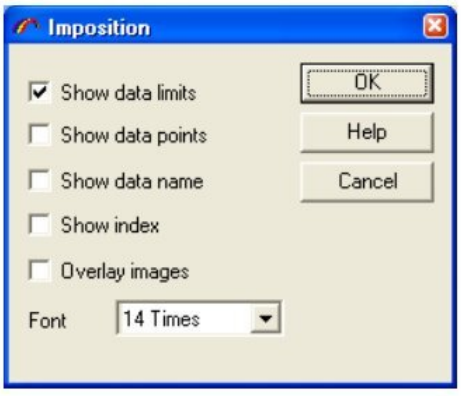
Available for: Video, Map, Score

8.17. Imposition

The Imposition dialog window allows information from the multidimensional spectral array Map and Score windows to be superimposed on the active video camera image.

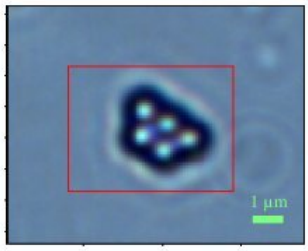
The display color is automatically assigned by LabSpec 5 to match the color of the object being superimposed.

To start the selected superimposition click **[OK]** and then activate a Map or Score window.



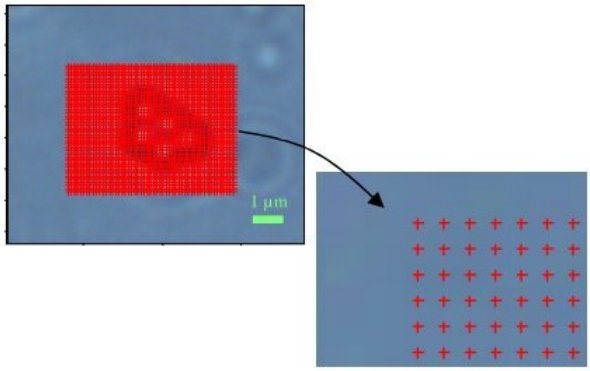
Show data limits

When "Show data limits" is ticked, the mapping area is superimposed on the video image.



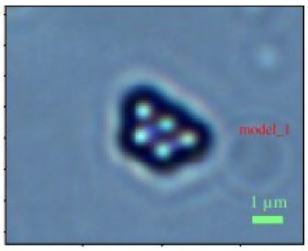
Show data points

When "Show data points" is ticked, the individual acquisition positions for the map are superimposed on the video image.



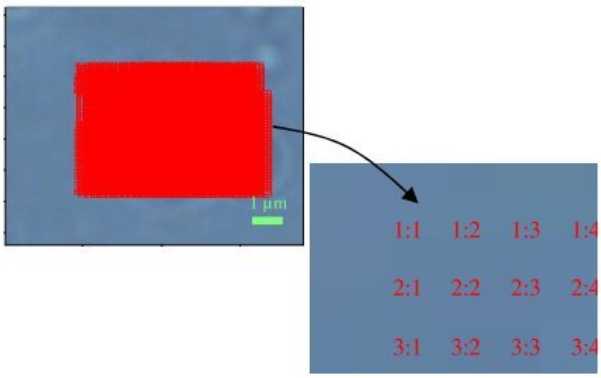
Show data name

When "Show data name" is ticked, the name of the image component is superimposed on the video image.



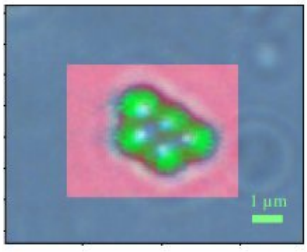
Show index

When "Show index" is ticked, the XY index of the individual acquisition positions for the map are superimposed on the video image.



Overlay images

When "Overlay images" is ticked the Map or Score image is superimposed on the video image. Note that the overlay mode in the Map/Score window will be reflected in the superimposition.



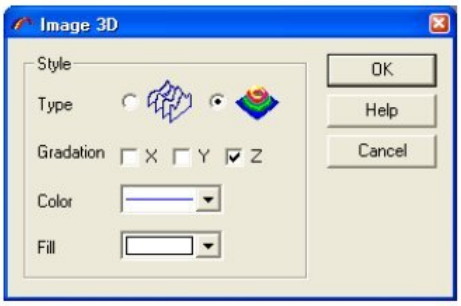
The superimposed image will always be displayed in True Color, whatever the palette setting in the Colors dialog window – see section 8.6, page 210.

Font
Select the font style for the data index and data name text display from the "Font" drop down box. Note that the font color is automatically assigned by LabSpec 5 to match the color of the object being superimposed.

Available for: *Video*

8.18. Image3D

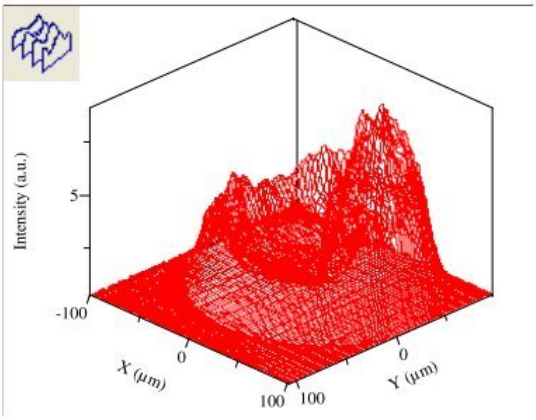
The Image3D dialog window allows control of formatting and display options for 3D images. It should be used in conjunction with the Colors dialog window (see section 8.6, page 210).



Type
Select the display mode from the following options:

- Line

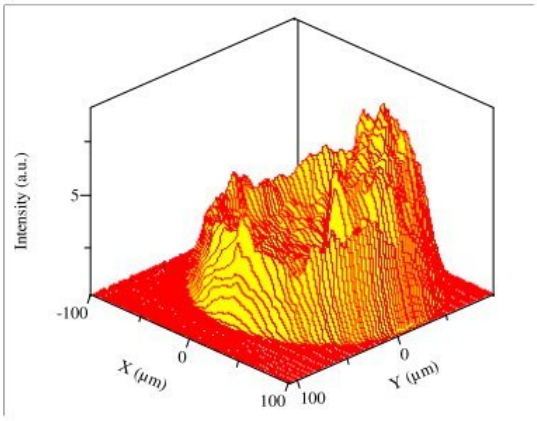
  The line color is selected from the "Line" drop down box.



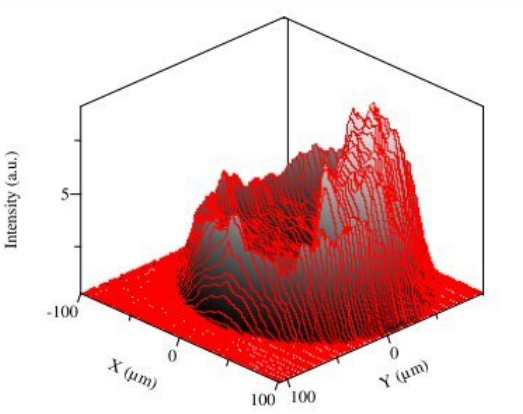
- Line and Fill

The line color is selected from the "Line" drop down box. The line display can be removed by setting the line color to "None".

When there is no "Gradation" selected (i.e., X, Y and Z gradation boxes are all unticked) the fill color is selected from the "Fill" drop down box. The example shown right is displayed without gradation.



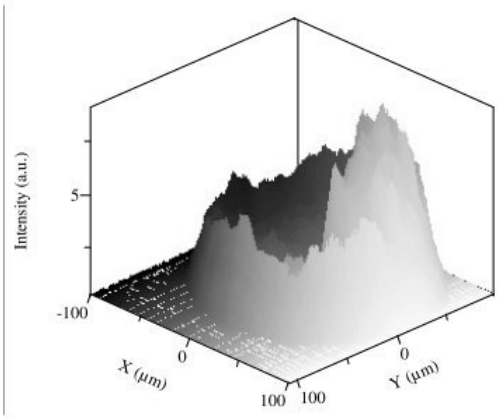
When "Gradation" is selected (i.e., one or more of the X, Y and Z gradation boxes are ticked) the graded fill color is set according to the palette selected in the Colors dialog window (see section 8.6, page 210). The example shown right is displayed with Z gradation using a greyscale palette.



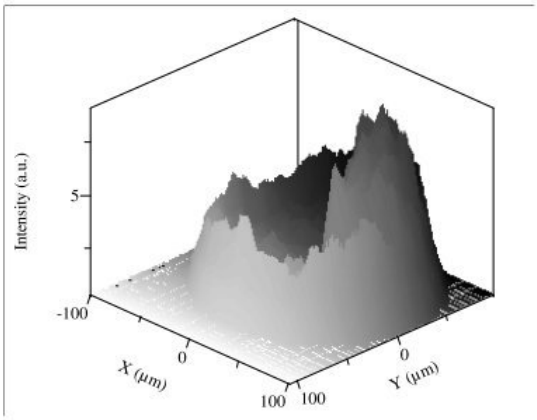
Gradation

Select the axis (or axes) direction for graded fill color:

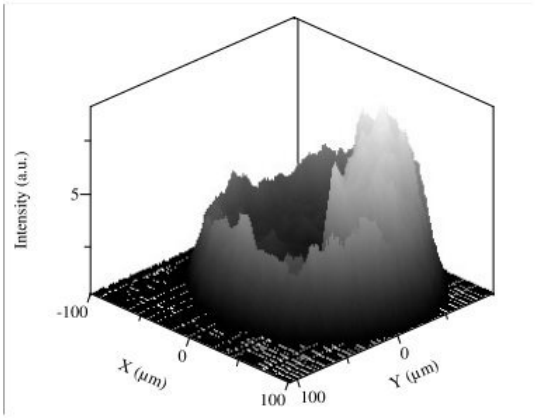
- X – graded fill from low X axis value to high X axis value.



- Y – graded fill from low Y axis value to high Y axis value.



- Z – graded fill from low Z (intensity) axis to high Z (intensity) axis.



Color
Select the line color from the "Color" drop down box.

Note that the Line and Width options in this drop down box are not active for 3D images.

Fill
Select the fill color from the "Fill" drop down box. The fill color selected in the "Fill" drop down box is only displayed when the "Line and Fill" type is selected, and no gradation option is selected (see above). If a gradation option is selected, the fill color is set according to the palette selected in the Colors dialog window (see section 8.6, page 210).

Note that the Fill option in this drop down box is not active for 3D images.

Available for: Video, SpIm, Map, Score (when displayed in 3D mode)

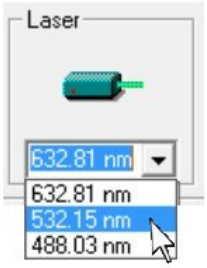
9. Control Panel

The Control Panel located at the bottom of the screen contains sections which are directly related to the hardware configuration of the instrument. This panel will *only* show sections for devices which are correctly installed and configured. The description below shows all of the standard sections, but be aware that some of these may not be visible in your software.

9.1. Laser

The drop down box lists all of the laser wavelengths available on the instrument.

Click on the drop down box to display the list, and then select the laser wavelength to be used.



On fully automated systems (such as LabRAM ARAMIS, automated LabRAM HR, or XploRA) the necessary hardware and optics will switch automatically.

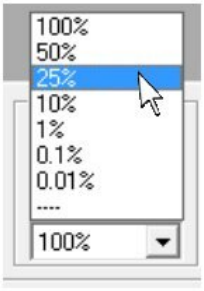
On manual systems (such as LabRAM 300, LabRAM 1B, manual LabRAM HR, U1000 or T64000) remember to change the optics necessary to use the selected laser. These optics include switching mirror(s), interference filter(s) and notch/edge filter(s).

9.2. Filter

Most systems have a number of neutral density filters available which can be used to reduce the laser power incident on the sample. Typically these are necessary if the sample is sensitive to the laser power, and burns/degrades when the full power is used. Note that the detected Raman signal is proportional to laser power, so the lower the laser power the longer your measurement will need to be to obtain a good quality Raman spectrum.

These filters are motorized, and will automatically be inserted into the laser path once selected.

The drop down box lists all of the filters available on the instrument. These will be displayed either in optical density (OD) or percentage (%). The following table shows the relationship between OD and % values.



OD	%
- - -	100% (no attenuation)
D0.3	50%
D0.6	25%
D1	10%
D2	1%
D3	0.1%
D4	0.01%

Click on the drop down box to display the list, and then select the laser wavelength to be used.

9.3. Hole

The confocal hole is used to define the spatial resolution and analysis volume of a measurement, and should be used in conjunction with correct choice of microscope objective and laser wavelength to fully optimise a measurement.

Typically the confocal performance of a system improves (i.e., the spatial resolution increases) as the confocal hole diameter decreases.

Confocal analysis
Confocal analysis means the measurement will be made with high spatial resolution, suitable for analysis of true microscopic particles and thin layers with dimensions in the range 500nm – 10µm. To analyze these types of samples the hole should be set to a small diameter. Note that in a confocal mode you are analysing less molecules (because the analysis volume is small), and the signal level you observe will decrease.

Macro or bulk analysis
For analysis of bulk powders and liquids, or any sample where high spatial resolution is not necessary, it is best to run the system in a non-confocal mode. By setting the hole diameter to a large value the spatial resolution will be low, and the analysis volume of the measurement will be increased. Note that in a non-confocal mode you are analysing more molecules (because the analysis volume is large), and the signal level you observe will increase.

Some instruments have fully adjustable holes, whilst others have fixed hole settings. The controls on these are different.

9.3.1. Control of fully adjustable hole

Type in the desired diameter of the hole, and press **<enter ↵ >** so that the value is registered.

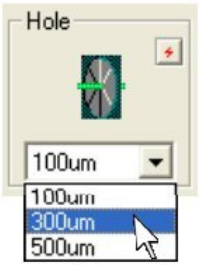
Click on the left hand arrow to send the hole to a closed position (0 µm diameter). 

Click on the right hand arrow to send the hole to its maximum position. 

Click on the initialization icon to send the hole to its reference position, and then back to the displayed diameter.

9.3.2. Control of fixed position hole

The drop down box lists all of the hole diameters available on the instrument.



Click on the drop down box to display the list, and then select the hole diameter to be used.

Click on the initialization icon to send the hole to its reference position, and then back to the displayed diameter.

9.4. Slit

The slit is used to define spectral resolution, and should be used in conjunction with correct choice of laser wavelength and diffraction grating. On most systems equipped with array detectors (such as a CCD or InGaAs array) the diffraction grating (see section 9.6.1, page 231) has a more significant effect on spectral resolution than the slit. The slit should only be adjusted once a suitable diffraction grating has been selected.

Typically the spectral resolution of a system will increase as the slit width is decreased. High spectral resolution (obtained with a narrow slit width) allows subtle changes in a spectrum to be confidently analyzed, and close lying peaks to be separated.

Some instruments have fully adjustable slits, whilst others have fixed slit settings. The controls on these are different.

9.4.1. Control of fully adjustable slit

Type in the desired diameter of the slit, and press **<enter ↵ >** so that the value is registered.

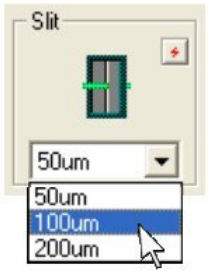
Click on the left hand arrow to send the slit to a closed position (0 µm width).

Click on the right hand arrow to send the slit to its maximum position.

Click on the initialization icon to send the slit to its reference position, and then back to the displayed width.

9.4.2. Control of fixed position slit

The drop down box lists all of the slit diameters available on the instrument.



Click on the drop down box to display the list, and then select the slit diameter to be used.

Click on the initialization icon to send the slit to its reference position, and then back to the displayed width.

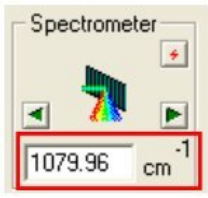
9.5. Spectrometer

The spectrometer control allows the wavelength (nm) or wavenumber (Raman shift, cm^{-1}) position to be selected. With an array detector (such as CCD or InGaAs array) the specified wavelength/wavenumber position lies at the center of the detector, and a certain range either side of this position will be detected. The precise range depends upon the spectrometer focal length, laser wavelength, and diffraction grating.

As an example, if the spectrometer position is set at $1500cm^{-1}$ then the lowest position could be $970cm^{-1}$, and the highest position could be $2030cm^{-1}$. The central position in the spectrum will be $1500cm^{-1}$.

The spectrometer position can be quickly controlled, allowing you to monitor Raman peaks in a specific area of the full spectrum. In most cases, the detected range will be less than the full Raman spectrum range, which is typically $100\text{-}4000cm^{-1}$. If you wish to acquire a spectrum across the full Raman range then you should use the Extended Range mode – see section 3.5.6, page 49.

Type in the desired central position of the spectrometer, and press **<enter ↵ >** so that the value is registered. The current units will be displayed next to the input box.



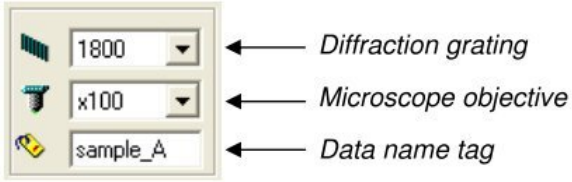
Click on the left hand arrow to send the spectrometer to the calibration 'Zero Order' position (0 nm).

Click on the right hand arrow to send the spectrometer to its maximum position. Note that the maximum position will depend upon the spectrometer, laser and diffraction grating.

Click on the initialization icon to send the spectrometer to its calibration position, and then back to the displayed position.

9.6. Options

The Options section allows you to specify the diffraction grating, microscope objective and data name tag.



9.6.1. Diffraction grating

The diffraction grating is the dispersing optical element within the spectrometer, which splits the spectrum into its constituent colours.

Each grating offers a certain level of dispersion (which affects the spectral resolution of the system) and wavelength coverage (which affects the spectrum intensity, and compatibility with particular laser wavelengths). Gratings are typically classified by a number of grooves per mm – for example, 300gr/mm (low resolution) or 3600gr/mm (high resolution). The higher the number the higher the achievable spectral resolution.

Each system is equipped with different diffraction gratings, which are chosen to take into account the available laser wavelengths and requirements for spectral resolution.

Most instruments have grating turrets, with multiple gratings attached. This allows you to quickly switch from one grating to another (for example, to switch from low resolution to high resolution analysis). The following list shows the standard maximum number of gratings available on the turret:

1 grating (fixed position):	HE spectrograph, Axial spectrograph
1 grating:	U1000, T64000
2 gratings (manual switching):	LabRAM 300 / 1B (and IR, IR^2 and INV configurations)
2 gratings (motorized switching):	LabRAM HR (and IR, IR^2 and INV configurations)
4 gratings (motorized switching):	LabRAM ARAMIS (and IR and IR^2 configurations), XploRA

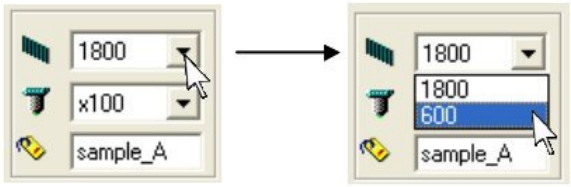
It is also possible to manually exchange diffraction gratings on the LabRAM HR base unit, U1000 and T64000. For example, a LabRAM HR could be equipped with three diffraction gratings in total. Two would be mounted on the motorized turret, and the third would be kept in a box by the system. One

of the gratings on the turret could be exchanged with the third grating if desired. Manually exchanging grating typically takes a few minutes to complete, and there is no optical realignment required.

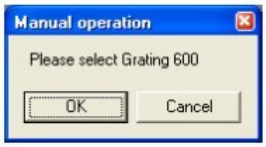
9.6.1.1. Selecting a grating (manual turret)

The drop down box lists all of the diffraction gratings available on the instrument.

Click on the drop down box to display the list, and then select the diffraction grating to be used.



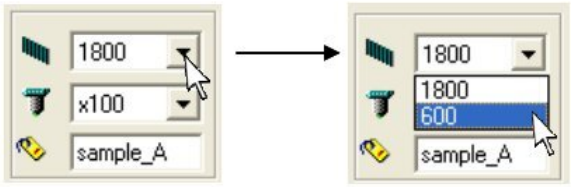
A message will prompt you to now manually select the desired diffraction grating. Typically this is done on the instrument using a push-pull bar, or other switching device. Once the diffraction grating is selected click **[OK]**.



9.6.1.2. Selecting a grating (motorized turret)

The drop down box lists all of the diffraction gratings available on the instrument.

Click on the drop down box to display the list, and then select the diffraction grating to be used. The diffraction grating will now be moved into position by the software.



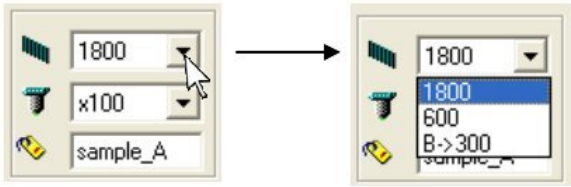
9.6.1.3. Selecting a grating for manual exchange

The drop down box lists all of the diffraction gratings available on the instrument.

Click on the drop down box to display the list. Gratings available for manual exchange are listed with either “A->” or “B->” in front of their identifier.

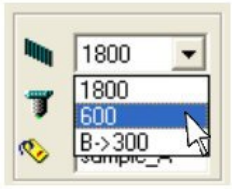
This implies that they are intended to be exchanged in the A position of the turret, or the B position of the turret. The first grating listed (in this case 1800) is in the A position. The second grating listed (in this case the 600) is in the B position.

This example shows that there is also a 300gr/mm grating which could be exchanged in the B



position. This means that it would replace the current 600gr/mm grating.

To replace the diffraction grating first select the diffraction grating you wish to replace, and send the spectrometer to Zero Order (see section 9.5, page 230). Now take out the current diffraction grating, and replace it with desired replacement diffraction grating.



Once the manual exchange is complete, now select the replacement diffraction grating in the drop down list.

A message box will appear prompting you to replace the grating. Click **[OK]**.

CAUTION: THE DIFFRACTION GRATING IS EXTREMELY FRAGILE AND EXPENSIVE. ON NO ACCOUNT SHOULD YOU TOUCH OR SCRATCH THE SURFACE OF THE GRATING. AS SOON AS IT IS REMOVED FROM THE INSTRUMENT IMMEDIATELY PLACE IT IN ITS PROTECTIVE CASING.

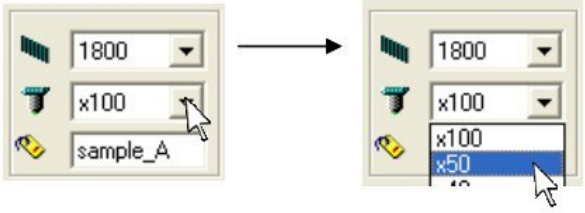
9.7. Microscope objective

On most systems the microscope is manually controlled, and objectives must be manually selected and exchanged. However, it is important that the correct objective is also selected in LabSpec 5 in order to have correctly scaled video images, and for Raman maps to be acquired over the correct part of the sample.

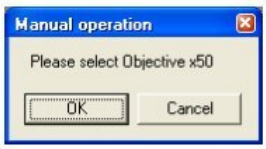
9.7.1.1. Selecting a microscope objective

The drop down box lists all of the objectives available on the microscope.

Click on the drop down box to display the list and then select the objective to be used.



A message will prompt you to now select the desired objective. Once the correct objective is selected on the microscope click **[OK]**.

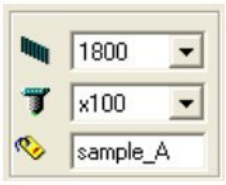


9.8. Data name tag

The data name tag is a prefix which is used for every newly acquired spectrum. In addition, it is used as the default file name during a File > Save As procedure.

If the data name is unchanged for multiple accumulations each new spectrum will be acquired with the same prefix, and then sequentially numbered.

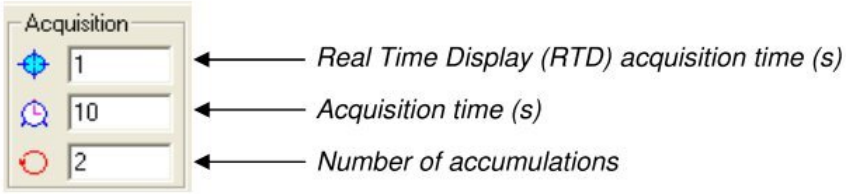
In the example shown to the right, the data name tag has been set as *sample_A*. Each spectrum will be acquired as *sample_A_1*, *sample_A_2* etc.



If the data name tag is left blank, as in this example, then spectra will be automatically labelled as *_1*, *_2* etc.

9.9. Acquisition

The Acquisition section allows the user to specify acquisition times (also called exposure times) for the Real Time Display, Spectrum Acquisition and Mapping Acquisition, and the number of accumulations (or averages) for each measurement.



9.9.1. Real Time Display (RTD) acquisition time

The Real Time Display (RTD) provides a continuous readout of the detector, and is useful to adjust the fine focus of the sample to optimise Raman signal, and to monitor whether a sample is degrading or burning. The RTD is started using the / icon – see section 4.5.1, page 95, for more details.

Normally the RTD acquisition time should be kept small in order to allow fast and continuous spectrum read out. Typical values are in the range 0.2s–2s.

To adjust the RTD acquisition time simply type the desired time (in seconds) into the box, and press **<enter ↵ >**.

9.9.2. Acquisition time

The acquisition time is the time taken to acquire a Raman spectrum. Typically the longer the acquisition time the better quality the resulting spectrum. The acquisition time set affects the Spectrum Acquisition (/ ; see section 4.5.3, page 96) and Mapping Acquisition (/ ; see section 4.5.4, page 96).

Note that for an extended range spectrum acquisition (see section 3.5.6, page 49) the acquisition time is the time taken for each spectral window within the full spectral range. For multidimensional spectral array measurements, the acquisition time is the time taken for each spectral window of each spectrum acquisition in the array.

Minimum acquisition times are in the order of 0.2s-0.5s for standard Raman spectra and mapping experiments, and <50ms for SWIFT ultra-fast Raman mapping experiments.

There is no maximum value for the acquisition time, since this will depend on the desired spectrum quality – however, the CCD detector will saturate at and above a particular signal level, so it is important that the acquisition time is not so large that saturation occurs. The saturation level depends on the specific detector installed on your system. Modern detectors generally saturate at approximately 65,000 counts, whereas older detectors may saturate at lower values (such as approximately 32,000 counts).

To adjust the acquisition time simply type the desired time (in seconds) into the box, and press **<enter ↵ >**.

9.9.3. Number of accumulations

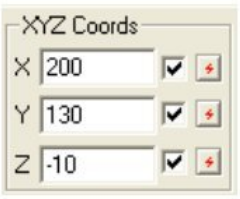
Acquiring multiple accumulations of data and averaging them results in improved spectrum quality. However, with array detectors such as CCDs and InGaAs arrays it is generally best to improve spectrum quality by first increasing the acquisition time. When the saturation point is reached, then it is worth increasing the number of accumulations.

Depending on the chosen mode of the spike (or cosmic ray) filter (see section 3.5.4.7, page 37) it is recommended to have a minimum of two accumulations so that the spike filter can detect and remove random spikes in the spectrum.

Data from multiple accumulations will either be averaged, or summed – the desired mode can be selected from Acquisition > Options (see section 3.5.4.6, page 37).

To adjust the number of accumulations simply type the desired number into the box, and press **<enter ↵ >**.

9.10. XYZ Coords



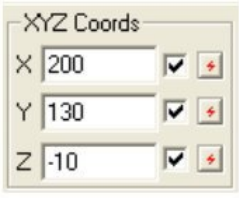
The XYZ Coords section shows the current position (in micrometers, µm) of installed XY and Z stages. It can be used for a number of functions:

- Move the stage by a set distance by inputting a value, rather than using the joystick
- Noting a reference position on a sample, and returning to that position as required
- Measuring distances moved by the stage
- Switching between different stage configurations (for example, motorized XY stage and DuoScan™).

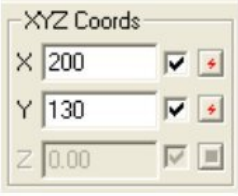
There are a number of variants of the XYZ Coords section, depending on the system configuration. The operation of each variant is described below.

9.10.1. Using XYZ Coords

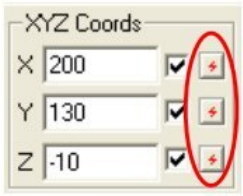
The current stage position (X, Y and Z coordinates) are shown in the respective boxes. The units are micrometers (µm). The tick boxes must be ticked for coordinate read out to be active for each axis.



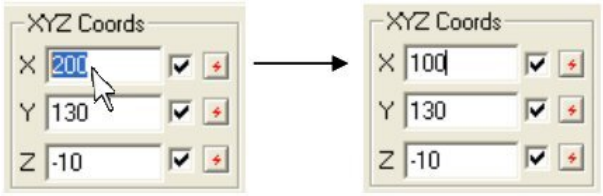
Note that if one of the stages is not present (for example, the Z stage) then it will be greyed out.



The current position coordinates can be set to 0 µm by clicking on the 'set current position as origin' icons for each axis.

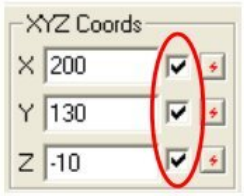


To move the stage to a specific position highlight the current value and type in the desired position (in micrometers, µm). Press **<enter ↵ >**. The stage will now move to the desired location.



CAUTION: MOVE THE STAGE WITH CAUTION, SINCE IT IS POSSIBLE TO DAMAGE THE SAMPLE, STAGE OR MICROSCOPE OBJECTIVE IF THE SAMPLE HEIGHT IS NOT CHECKED. THERE IS PARTICULAR RISK WHEN THE Z STAGE IS USED WITH HIGH MAGNIFICATION OBJECTIVES.

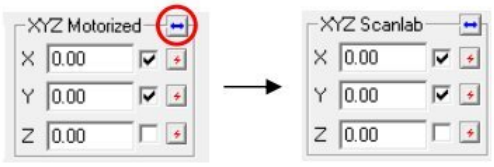
The coordinate read out and control can be deactivated for each axis independently by unticking the respective tick box.



9.10.2. Using XYZ Coords with multiple stages

The main control of XYZ Coords when multiple stages are configured and active is identical to that outlined above (see section 9.10.1, page 236). Note that the name of the XYZ stage is displayed in this section – typically the stages will be "XYZ Motorized" for a standard motorized stage, and "XYZ Scanlab" for the DuoScan™ stage (see section 4.5.10, page 110)

Click on the 'switch stage' icon, to select the desired stage.



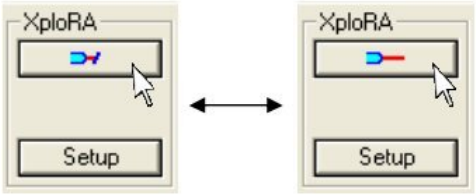
9.11. Instrument Setup

The Instrument Setup section is only present for the XploRA™ or LabRAM ARAMIS systems. It allows control of the laser beam (on/off) and other hardware options (such as polarizors, trinocular head, macro chamber etc).

9.11.1. Control of Laser Beam (for XploRA™)

On the XploRA™ system the selected laser (see section 9.1, page 227) can be turned on or off using the laser toggle switch.

Click on the toggle switch to turn the laser on and off.

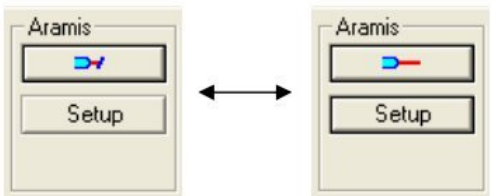


Laser off **Laser on**

9.11.2. Control of Laser Beam (for LabRAM ARAMIS)

On the LabRAM ARAMIS system the mechanical shutter for the selected laser (see section 9.1, page 227) can be opened or closed using the toggle switch. When the shutter is open the sample will be exposed to the laser (if the laser is turned on and active).

Click on the toggle switch to open or close the shutter.

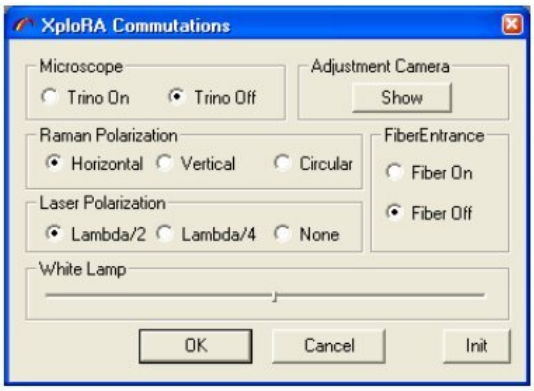


Laser shutter closed **Laser shutter open**

9.11.3. Control of Hardware Options (for XploRA™)

The XploRA Setup window allows control of the following optional items:

- Trinocular head on the microscope
- Raman polarizors
- Laser polarizors
- Internal adjustment camera
- White lamp illuminator for the internal adjustment camera
- Fibre entrance

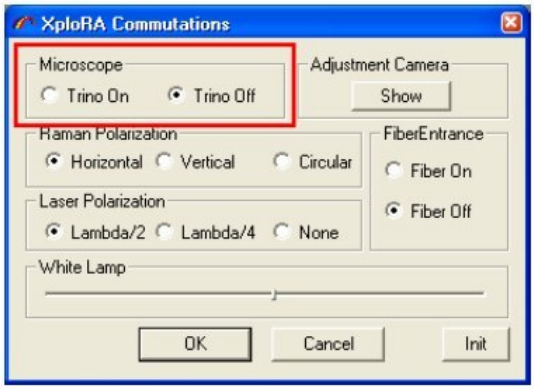


Please note that depending on the configuration of your system certain items in the Setup window may be greyed out.

The XploRA Setup window is opened by clicking on the Setup button.

9.11.3.1. Microscope

Select {Trino On} or {Trino off} to control the optical path to the trinocular head.



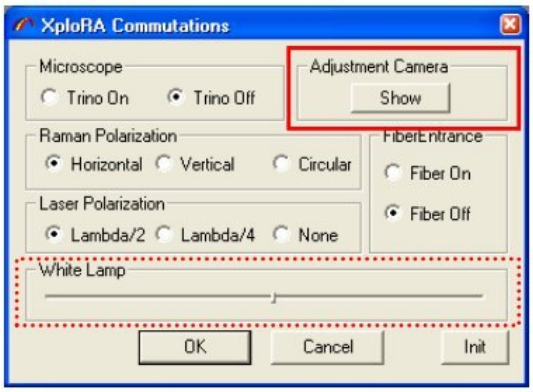
Trino On
Microscope eye pieces and top camera are active; Raman path is inactive.

Trino Off
Raman path is active; microscope eye pieces and top camera are inactive.

9.11.3.2. Adjustment Camera

Click on **[Show]** to activate the internal adjustment camera and to switch the microscope to "Trino Off" mode.

This camera is useful to visualize the laser spot on the sample. White light illumination can be provided using the "White Lamp" slider bar to control an internal white LED, or by using the standard XploRA™ microscope illumination options.



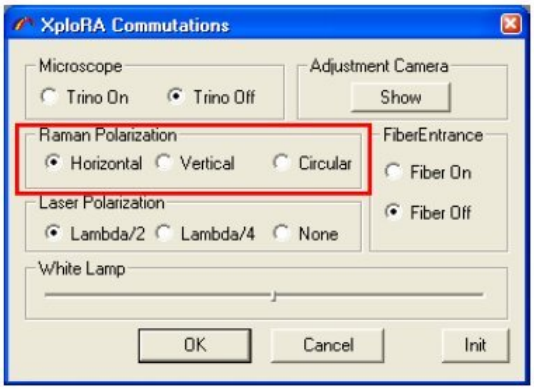
9.11.3.3. Raman Polarization

Select {Horizontal}, {Vertical} or {Circular} to insert a polarizor element into the Raman beam path to control the polarization of the analyzed Raman signal.

Horizontal
Only Raman signal which is horizontally polarized will be detected.

Vertical
Only Raman signal which is vertically polarized will be detected.

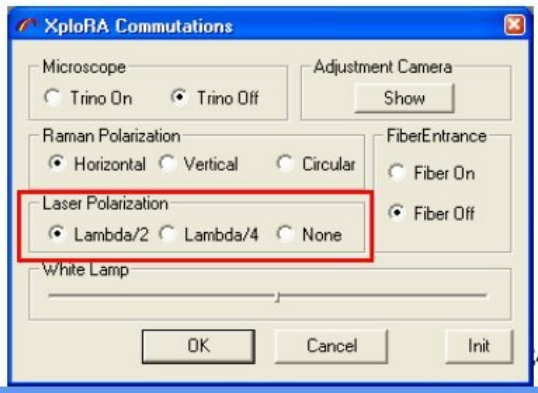
Circular
All Raman signal will be converted to circularly polarized light prior to detection. Most spectrometers have different sensitivities for horizontally and vertically polarized light. By converting the signal from horizontal/vertical polarization to circular polarization the difference in spectrometer performance will be removed, and all light will be detected in exactly the same way.



9.11.3.4. Laser Polarization

Select {Lambda/2}, {Lambda/4} or {None} to insert a polarizor element into the laser beam path to adjust the polarization of the laser beam.

Lambda/2 (or $^{\lambda}/_{2}$)
Rotates the laser beam polarization by 90°.

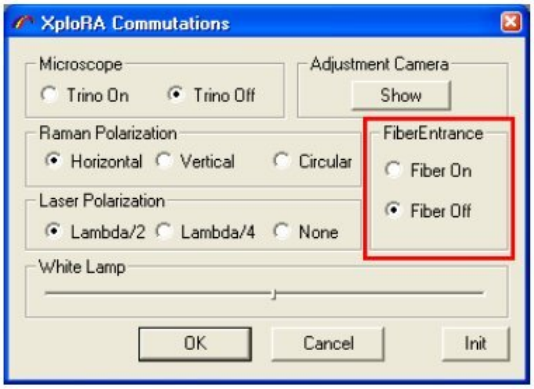


Lambda/4 (or $\lambda/4$)
Converts the laser beam polarization from linearly polarized to circularly polarized.

None
Leaves the laser beam with its intrinsic linear polarization.

9.11.3.5. Fiber Entrance

Select {Fiber On} or {Fiber Off} to select whether the Raman signal is detected from the microscope, or from a separate fiber optically coupled remote Raman probe head, such as a SuperHead.

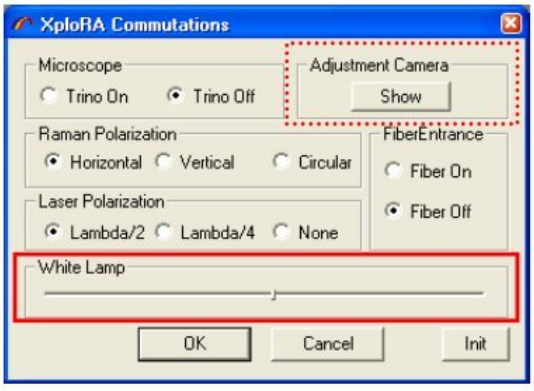


Fiber On
In this mode, only Raman signal which is delivered via a fibre optic to the FC connector on the underside of the XploRA™ platform will be detected. You should select this mode if you wish to work with a remote Raman probe.

Fiber Off
In this mode, Raman signal which originates from the microscope will be detected. You should select this mode if you wish to make 'standard' microscope measurements with the XploRA™.

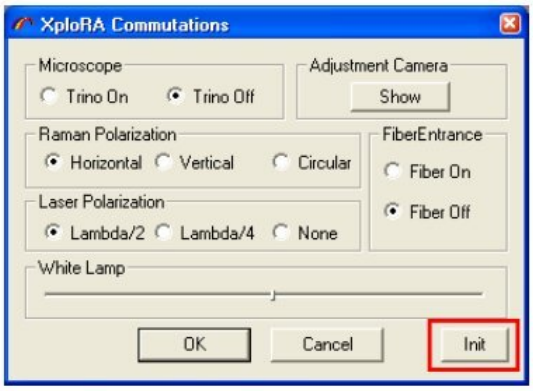
9.11.3.6. White Lamp

The White Lamp slider controls the intensity of an internal white LED to illuminate the sample when it is visualized using the internal Adjustment Camera.



9.11.3.7. Init

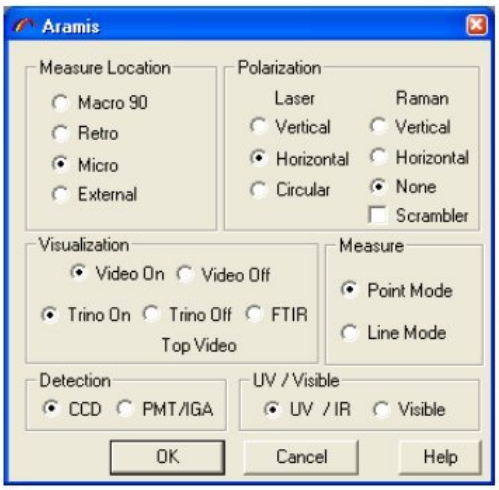
The **[Init]** button will re-initialize all of the XploRA motors, and confirm that they are at their correct positions.



9.11.4. Control of Hardware Options (for LabRAM ARAMIS)

The LabRAM ARAMIS Setup window allows control of the following optional items:

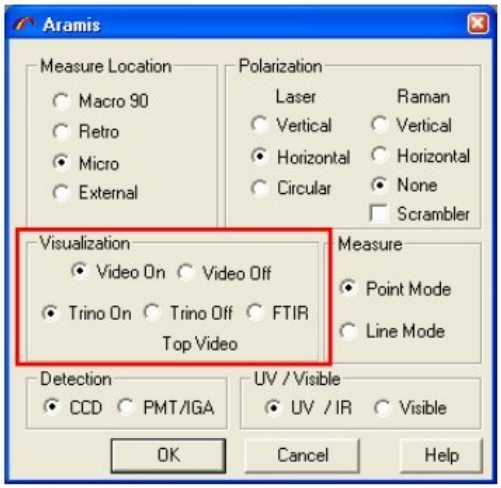
- Measurement location (microscope, fibre optic probe, macro chamber)
- Laser and Raman path polarizors
- Trinocular head on the microscope
- Multiple detectors
- "Point Mode" and "Line Mode" optics
- UV/IR and visible dual path optics



Please note that depending on the configuration of your system certain items in the Setup window may be greyed out.

The LabRAM ARAMIS Setup window is opened by clicking on the Setup button.

9.11.4.1. Visualization



Select {Video On} or {Video Off} to control the internal camera optics.

Video On
Optics for internal camera and reflected white light illumination are in the light path; Raman path is inactive. This mode is used to visualize the sample and acquire white light images of the sample using the internal camera.

Video Off
Raman path is active; optics for internal camera and reflected white light illumination are out of the light path. This mode is used to acquire a Raman spectrum.

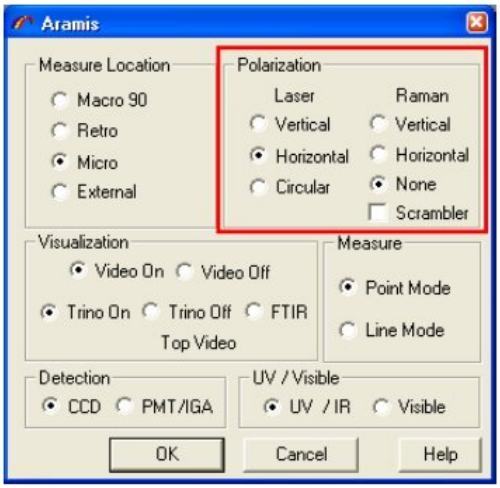
Select {Trino On}, {Trino Off} or {FTIR} in the "Top Video" section to control the optical path to the optional trinocular head and FTIR module

Trino On
Microscope eye pieces and top camera are active, with illumination beam splitter in place; Raman path, internal camera optics and FTIR module are inactive.

Trino Off
Raman path and internal camera optics are active; microscope eye pieces, top camera and FTIR module are inactive.

FTIR
FTIR module is active; Raman path and internal camera optics are inactive; microscope eye pieces and top camera are active for transmitted white light illumination only, and inactive for reflected white light illumination.

9.11.4.2. Polarization



Select the required polarizor element to control the polarization of the laser beam and the analysed Raman signal.

Laser - Vertical
Allows the laser beam to be linearly polarized in a vertical orientation.

Laser - Horizontal
Allows the laser beam to be linearly polarized in a horizontal orientation.

Laser - Circular
Converts the laser beam polarization from linearly polarized to circularly polarized.

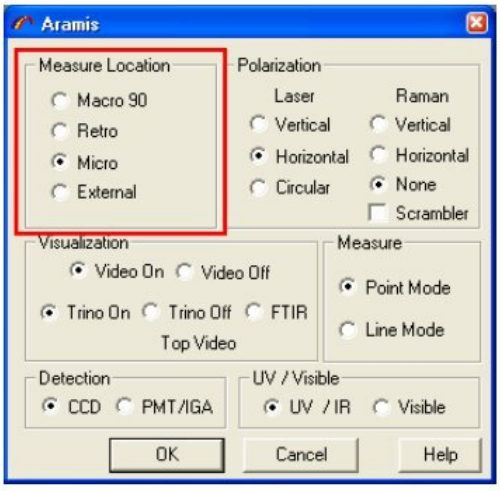
Raman - Vertical
Only Raman signal which is linearly polarized in a vertical orientation will be detected.

Raman - Horizontal
Only Raman signal which is linearly polarized in a horizontal orientation will be detected.

Raman - Circular
All Raman signal will be converted to circularly polarized light prior to detection.

Raman – Scrambler
The polarization of the Raman signal will be scrambled (removed) prior to detection.

9.11.4.3. Measure Location



Select the required measurement location on the system, including the microscope, macro chamber and external fibre optically coupled probes.

Macro 90
In this mode, Raman signal which originates from the macro chamber in a 90° orientation will be detected. You should select this mode if you wish to analyse bulk materials in the macro chamber using a 90° orientation.

Retro
In this mode, Raman signal which originates from the macro chamber in a 180° back scattering (retro) orientation will be detected. You should

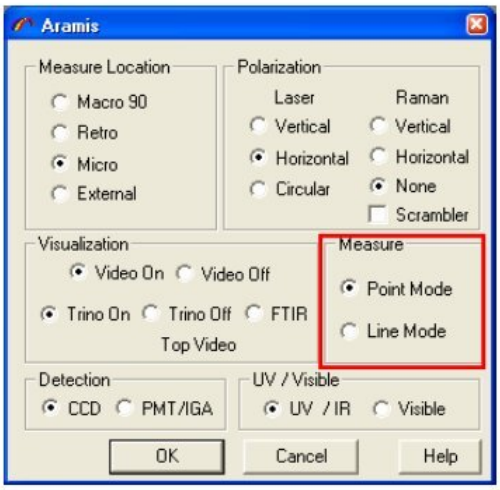
select this mode if you wish to analyse bulk materials in the macro chamber, using a back scattering geometry.

Micro
In this mode, Raman signal which originates from the microscope will be detected. You should select this mode if you wish to make 'standard' microscope measurements with the LabRAM ARAMIS.

External
In this mode, Raman signal which originates from an external location will be detected, using the fiber optical input. The light is passed directly from the fiber optical input into the spectrometer, bypassing the microscope optics. You should select this mode when working with SuperHead fiber optic remote probe heads

9.11.4.4. Measure

Select {Point Mode} or {Line Mode} to control the optics for standard spot analysis or confocal line scan analysis.



Point Mode
Sets up the measurement for standard spot (or point) analysis. You should select this mode if you wish to make 'standard' measurements with a 0.5-10µm laser spot.

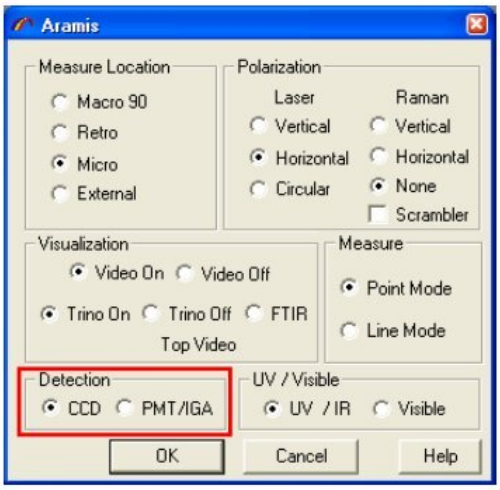
Line Mode
Sets up the measurement for confocal line scan analysis. You should select this mode in combination with the LineScan scanning mirror controller, which allows the laser spot to be rapidly rastored across the sample. This is useful for acquiring an average spectrum from along the entire line, and for fast Raman mapping applications.

9.11.4.5. Detection

Select {CCD} or {PMT/IGA} to control the optics for multiple detectors mounted on the LabRAM ARAMIS.

CCD
In this mode the standard CCD detector will be used for the analysis.

PMT/IGA
In this mode the detector mounted on the second port will be used for the analysis. Typically this detector will either be a photomultiplier tube (PMT), InGaAs array (IGA) or a second specialized CCD.

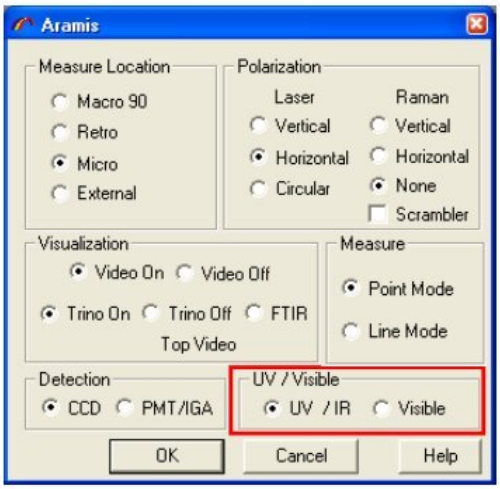


9.11.4.6. UV / Visible

Select {UV / IR} or {Visible} to control the dual path optics in the LabRAM ARAMIS, which allow optimised detection in either the UV/IR or visible spectral regions.

UV / IR
In this mode, mirrored optics are used which are optimised for UV and/or infra-red (IR) analysis.

Visible
In this mode achromatic lense optics are used which are optimised for standard visible analysis.



10. Appendix: Manual Wavelength Calibration of LabRAM Systems

The procedure outlined below can be used to manually calibrate the LabRAM systems (including LabRAM 300, LabRAM 1B, LabRAM HR, LabRAM ARAMIS, LabRAM INV, LabRAM IR and LabRAM IR^2).

The full calibration procedure uses two software parameters

- ZERO: for calibration of the diffraction grating's zero order peak position (0 nm)
- KOEFF: for calibration of the Raman peak position

Note that this calibration procedure is a diffraction grating calibration, and should be repeated for all diffraction gratings on the system. The software will retain individual calibration values for each diffraction grating.

A full calibration of a system with multiple lasers requires one of the lasers to be designated as the 'reference laser' – the wavelength of this laser is assumed to be constant, and known. The remaining laser wavelengths are then calibrated relative to the reference laser.

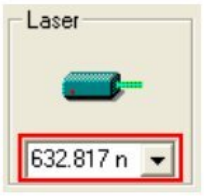
Before performing a manual calibration, ensure that all lasers which are to be used for the calibration have been turned on and left to warm up for a minimum of 15-30 minutes. This is essential to ensure the laser wavelength has stabilised.

10.1. Calibration of Zero Order Position (ZERO)

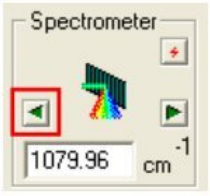
Insert the silicon (Si) calibration sample underneath the microscope, and focus on it in the normal way, using a 50x or 100x magnification objective.

Selecting the diffraction grating you wish to calibrate, using the Diffraction Grating drop down box in the control panel.

Select the reference laser from the Laser drop down box in the Control Panel.



Send the spectrograph to zero order (0 nm) using the icon in the Spectrometer section of the hardware toolbar.



Turn on the reference laser, and acquire a spectrum at zero order (0 nm) using the real time display (RTD) function. Ensure the detector is not saturated - if the signal is too intense, insert a filter in the laser path, reduce the confocal hole diameter, or decrease the acquisition time.

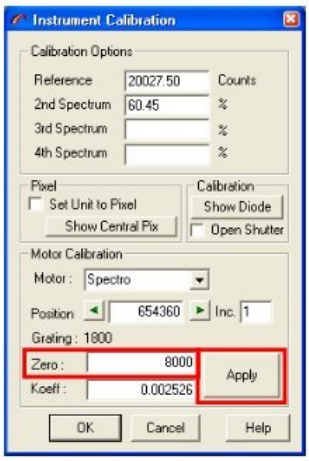

Stop the spectrum readout by clicking **[STOP]**.

Zoom in on the peak, using the Zoom icon in the Graphical Manipulation toolbar.

Use the cursor to locate the center of the zero order peak.

If the peak is within ±1 data point of 0 nm, the calibration is acceptable. If the peak is at a position greater than ±1 data point from 0 nm the calibration must be adjusted.

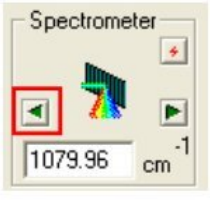
To do this click on Setup > Instrument Calibration to open the Calibration dialog window.



Adjust the ZERO parameter and then click the **[Apply]**. You should see the peak move. Continue to adjust the ZERO value until the band is within ±1 data point of 0 nm.

Click **[OK]** to save the calibration values for the current grating and close the Calibration dialog window.

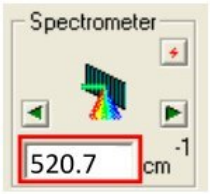
Resend the spectrograph to zero order and retake the spectrum. This will make sure that your calibration changes have taken effect. Check the zero order position again and if necessary, make further adjustments (change ZERO, resend to zero, check position etc) until the position is acceptable.



NOTE: The ZERO parameter should be changed in small increments e.g. ±5 at a time.

10.2. Calibration of Raman Peak (KOEFF)

Move the spectrometer to 520.7 cm^{-1} to analyse the silicon (Si) first order Raman peak.



Acquire a spectrum of the silicon (Si) sample using the real time display (RTD) function. Ensure the detector is not saturated - if the signal is too intense, insert a filter in the laser path, reduce the confocal hole diameter, or decrease the acquisition time.

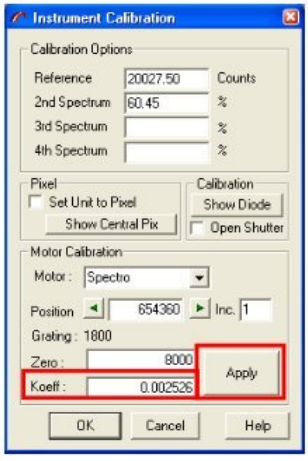
Stop the spectrum readout by clicking **[STOP]**.

Zoom in on the peak, using the Zoom icon in the Graphical Manipulation toolbar.

Use the cursor to locate the center of the 520.7 cm^{-1} silicon (Si) peak.

If the peak is within ±1 data point of 520.7 cm^{-1}, the calibration is acceptable. If the peak is at a position greater than ±1 data point from 520.7 cm^{-1} the calibration must be adjusted.

To do this click on Setup > Instrument Calibration to open the Calibration dialog window.



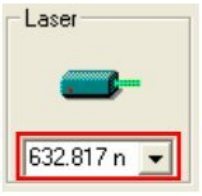
Adjust the KOEFF parameter and then click **[Apply]**. You should see the peak move. Continue to adjust the KOEFF value until the band is within ±1 data point of 520.7 cm^{-1}.

Click **[OK]** to save the calibration values for the current grating and close the Calibration dialog window.

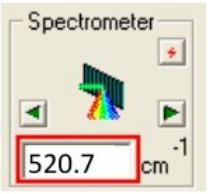
10.3. Calibration of Additional Lasers

The process outlined above is used to calibrate the diffraction grating, using the reference laser. Additional lasers must be calibrated using the following procedure – each laser must be calibrated individually, using this procedure.

Select the laser to be calibrated from the Laser drop down box in the Control Panel.



Move the spectrometer to 520.7 cm^{-1} to analyse the silicon (Si) first order Raman peak.



Acquire a spectrum of the silicon (Si) sample using the real time display (RTD) function. Ensure the detector is not saturated - if the signal is too intense, insert a filter in the laser path, reduce the confocal hole diameter, or decrease the acquisition time.

Stop the spectrum readout by clicking **[STOP]**.

Zoom in on the peak, using the Zoom icon in the Graphical Manipulation toolbar.

Use the cursor to locate the center of the 520.7 cm^{-1} silicon (Si) peak.

If the peak is within ±1 data point of 520.7 cm^{-1}, the calibration is acceptable. If the peak is at a position greater than ±1 data point from 520.7 cm^{-1} the calibration must be adjusted.

To do this manually adjust (by left clicking and typing) the laser wavelength displayed in the Laser drop down box in the Control Panel and press **<enter ↵ >**.

Repeat this laser calibration procedure for all other lasers which are to be calibrated.

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